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**Applications of spice extracts and other
hurdles to improve microbial safety and shelf-
life of cooked, high fat meat products (doner
kebab)**

by

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Applications of spice extracts and other hurdles to improve microbial safety and shelf- life of cooked, high fat meat products (doner kebab)

Abstract

There is a growing demand for safe and convenient meat products. The effect of natural spice extracts incorporated with other hurdles for controlling pathogenic bacteria and extending the shelf life of RTE doner kebab were investigated. A comprehensive literature review was undertaken to establish the status of microbial risk, use of additives, knowledge on oxidative deterioration and HACCP associated with meat products. The *in vitro* antioxidant and antibacterial activities of spice extracts were screened and compared. Cinnamon, clove, and sumac alcoholic extracts demonstrated strong antimicrobial effect, however, rosemary proved effective as antioxidant in a lamb fat model. An accelerated shelf life study on a model system indicated that storage temperature was the most critical factor affecting lipid oxidation, which was effectively delayed by vacuum packaging and rosemary extracts.

The effects of spice extracts, packaging and storage time on physiochemical, microbiological, and sensory attributes of doner kebab were evaluated. Application of rosemary and cinnamon extracts significantly reduced TVC, inhibited LAB, and retarded lipid oxidation rate. Sensory evaluation by a consumer panel indicated that only taste and spiciness perception was significantly different between treatments. A challenge test against *Listeria monocytogenes* showed significant differences between control and spice treatments at day 28. Strong inhibitory effects were associated to high levels of cinnamon particularly when applied after cooking. The effect of heat treatment and sumac (*Rhus coriaria*) on *Bacillus cereus* and *Clostridium perfringens* inactivation was evaluated on a doner kebab prototype. Addition of sumac significantly reduced *D*-values and *z*-values for both organisms in comparison to the control.

The investigation of the effect of spice extracts, and environmental conditions on changes in growth kinetic parameters for *L. monocytogenes* and *Salmonella* Typhimurium showed that spice extracts are highly significant. For both microorganisms, μ_{max} was reduced as salt and spice concentrations increased, and pH levels decreased. This study shows that spice extracts incorporated with other hurdles can help to maintain safe and good quality RTE doner kebab.

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List of abbreviations

| <u>Abbreviations</u> | <u>Glossary of terms used</u> |
|-----------------------------|--|
| % | Percentage |
| µl | Microliter |
| ΔE^{lab} | Colour difference |
| AC | After cooking |
| ANOVA | Analysis of variance |
| AOCS | American Oil Chemist Society |
| AOM | Active oxygen methods |
| APC | Aerobic Plat Count |
| ATP | Adenosine triphosphate |
| AU | Activity Unit |
| a_w | water activity |
| <i>B. c</i> | <i>Bacillus cereus</i> |
| <i>B. s</i> | <i>Bacillus subtilis</i> |
| BC | Before cooking |
| BCA | Bacillus cereus agar |
| BP | Baird–Parker agar |
| BPA | Black Pepper Aquaresin |
| BPO | Black Pepper Oleoresin |
| C.V | Coefficient of determination |
| CA | Cinnamon Aquaresin |
| CFU | Colony Forming Unit |
| <i>Cl. p</i> | <i>Clostridium perfringens</i> |
| CLA | Clove aquaresin |
| CLO | Clove Oleoresin |
| CO | Cinnamon Oleoresin |
| DIZ | Diameter of inhibition zone |
| <i>E. coli</i> | <i>Escherichia coli</i> |
| Eos | Essential oil |
| F | Process lethality |
| FSA | Food Standards Agency |
| GDA | Guideline daily allowance |
| GLM | General linear model |
| GLM | General linear model |
| GRAS | Generally recognized as safe |
| HACCP | Hazard Analyses and Critical Control Point |
| HPP | High Pressure Processing |
| HT | Hurdle technology |
| <i>L. in</i> | <i>Listeria innocua</i> |
| <i>L. m</i> | <i>Listeria monocytogenes</i> |
| <i>Lb. s</i> | <i>Lactobacillus sake</i> |

| | |
|----------------|--|
| LCL | Lower count limit |
| LSA | Listeria selective agar |
| LSD | Leas Significant Differences |
| MA | Spearmint Aquaresin |
| MAD | Malondialdehyde |
| MAP | Modified Atmosphere Pack |
| MIC | Minimum inhibitory concentration |
| Min | Minute |
| MRD | Maximum recovery diluent |
| MRS | DE MAN, ROGOSA, SHARPE |
| Mumax | Maximum Specific Growth Rate |
| N ₀ | Initial Bacterial Density |
| Nmax | Final Bacterial Density |
| OPSP | Perfringens Agar |
| OSI | Oil stability index |
| P | Statistical probability |
| PCR | Polymerase chain reaction |
| PHLS | Public Health Laboratory Service |
| PMP | Pathogen modelling program |
| ppm | Parts per million |
| <i>Ps. a</i> | <i>Pseudomonas aeruginosa</i> |
| RA | Rosemary Aquaresin |
| RBCA | Rose Bengal Chloramphenicol Agar |
| RE | Relative Efficacy |
| RMS | root mean square error |
| RO | Rosemary Oleoresin |
| RSA | Response surface analysis |
| RSS | Residual Sum of Squares |
| RTE | Ready to Eat |
| RVS | Rappaport-Vassiliadis Soya Peptone broth |
| <i>S. t</i> | <i>Salmonella</i> Typhimurium |
| SE | Standard error |
| SA | Sumac Water Extract |
| SEM | Scanning electron microscopy |
| SI | Stability Unit |
| SO | Sumac Hydro Alcoholic Extract |
| Staph.a | Staphylococcus aureus |
| TBA | Thiobarbituric Acid |
| TBARS | Thiobarbituric Acid Reactive Substances |
| TBX | Tryptone Bile Agar |
| t-d | Doubling time |
| TDC | Thermal death curve |
| TEM | Transmission electron microscopy |
| TEP | 1,1,3,3-Tetraethoxypropane |
| t-lag | Lag time |
| UCL | Upper count limit |
| VP | Vacuum Pack |
| VRBGA | Violet Red Bile Glucose Agar |
| WOF | Warmed Over Flavour |
| XLD | Xylose lysine deoxycholate agar |

Dedication

To my parents with my love

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Chapter 1

1. General Introduction, aim and objective and hypothesis

1.1 Introduction

Meat is one of the most nutritive materials, composed of proteins, fat and some essential elements, spoils quickly and becomes hazardous due to microbial growth; unless correctly stored, processed, packaged, and distributed (McDonald and Sun 1999, Shimoni and Labuza 2000). In several countries and under certain circumstances diseases caused by contaminated meat can lead to serious consequences (Angelillo et al. 2000). According to the World Health Organization (2002), per annum 10^9 of people fall ill and may die as a result of eating contaminated meat with food-borne bacteria and their toxins.

The presence of pathogens in meat products at low concentration is undesirable and considered as a main cause of food-borne disease world-wide (Buchanan and Whiting 1996). Therefore, when considering food safety we need to focus on pathogenic bacteria such as: *Salmonella spp.*, *Staphylococcus aureus*, *Campylobacter spp.*, *Escherichia coli*, *Listeria monocytogenes*, *Clostridium perfringens*, *Clostridium botulinum*, and *Bacillus cereus* (Robinson et al. 1998, Roberts 2000) because they are responsible for most cases of food-borne illness and food poisoning (International Commission on Microbiological Specifications for Foods 2005).

The symptoms associated with food poisoning and food-borne illness are those related to the gastrointestinal tract, including nausea, vomiting, diarrhoea and abdominal pain. The young, elderly, immune compromised, and those who are undergoing radiotherapy or chemotherapy are most at risk from all infectious

diseases (Pepin et al. 1997, Phillips 1998). Although the significance of food-borne pathogens on public health is high, it has also economic consequences for individuals, families, communities, businesses, and countries (World Health Organization 2002).

Recently, consumers are increasingly concerned about the safety and the quality of meat products. Thus, the validation of shelf-life is important for ensuring the microbiological safety of perishable food, specifically, food which is ready to eat and/or supports the growth of pathogens such as *L. monocytogenes* (Food Safety Authority of Ireland 2011).

Regulatory inspection and sampling regimes are employed as traditional approaches to meat safety and quality. However, these systems cannot absolutely guarantee consumer protection unless 100% inspection and sampling is used, which would be impractical in the meat industry for a variety of economic and logistic reasons (Armitage 1997).

The shelf life of meat products is considerably affected by the combination and interaction of intrinsic and extrinsic factors including pH, water activity (a_w), redox potential, storage temperature, and packaging. Some of these factors have a significant impact not only on the survival and growth of pathogenic and spoilage bacteria but also on the chemical and physical characteristics of meat products. Establishing meat product characteristics help the food processor and other stakeholders in the food chain to identify the type of microorganisms, including pathogens that may survive and grow in their products (Food Safety Authority of Ireland 2011, U.S. Food and Drug Administer 2001).

Although preservation methods (vis. temperature (high or low), packaging, preservatives, drying, curing and high hydrostatic pressure) have been employed world-wide to improve the quality and safety of meat products, cases

of food poisoning and food deterioration continue to rise in most countries (Ananou 2007). Accordingly, advancement of microbiological techniques along with other disciplines is required to develop the production, conduct a risk profile of meat products in particular RTE meat and, or to initiate a qualitative risk assessment (Luber et al. 2011).

As reported by Baird Parker and Kilsby (1987) traditional microbiological methods need intensive time and are usually only applicable to specific conditions. Thus, software using mathematical models such as Pathogen Modelling Program, Growth Predictor, and MicroFit could conquer these limitations, offer a cost effective means and become a very valuable part of meat production and the prevention of both food borne disease and food deterioration (McMeekin et al. 2010).

Recently, the use of natural safe plant ingredients in meat products such as spices and herbs is gaining impetus, due to consumer's demands toward healthy food free from synthetic additives. In addition, plant extracts have been shown to have a broad antimicrobial and antioxidant effect in meat products (Tajkarimi et al. 2010). Therefore, the application of spice extracts could improve the safety and the quality in high fat meat products specially if applied in combination with preservation techniques, thus becoming an additional mild deterioration barrier in the application of the so called hurdle technology.

Kebabs and similar traditional Middle Eastern meat products had been identified as sources of these infections. Recently reported data by Health Protection Agency (HPA) (2004), on the microbiological status of doner kebabs at point of sale pointed out that 16% of doner kebab samples were acceptable, and 4% were of unsatisfactory. The unsatisfactory results were caused by high counts

of *Bacillus* spp. at a level of 10^5 CFUml⁻¹ or more, *E. coli* and *Listeria* spp. (not *L. monocytogenes*) in excess of 10^2 CFUml⁻¹.

Local authority regulatory and related services in the UK (2009), reported that doner kebab contains over 1000 calories, which is over half the Guideline Daily Amount (GDA) for women and 40% for men and is also marked with three red traffic lights for fat, saturated fat, and salt.(Food Standards Agency 2007a). Additionally, there are few studies in the Middle East to evaluate the hygienic and quality status of doner kebab. Thus, information on chemical composition, microbiological status and sensory property for this product would be very useful.

Aim and objectives

The overall aim of the project is to apply a novel detection method to obtain safe and good quality high fat meat products such as doner kebab and ready to eat doner kebabs. This will be done by establishing the potential effect of heat treatment to control pathogenic bacteria and reduce risk. The optimization of antibacterial and antioxidant effect of spice extracts application to doner kebab to obtain a wholesome and safe product to meet consumers demand. Carry out risk assessment considering both traditional practices and novel technologies that allow convenience and extension of the shelf life of cooked products, such as vacuum packing and modified atmosphere packaging and finally using mathematical modelling as a developed microbiological technique to conduct the risk associated with this type of meat product. The schematic outline of the thesis is summarized in Figure. 1.1.

The main hypotheses

- The combination of spices with vacuum packaging (VP) and modified atmosphere packaging (MAP) would be effective for extending the shelf life of ready-to-eat doner kebab by retarding lipid and colour oxidation, maintaining sensory quality and preventing the effects of spoilage bacteria.
- Application of spice extracts in combination with other hurdle techniques would reduce the risk from pathogenic bacteria in high fat meat products.
- The identification of the extent of heat penetration in doner kebab will be useful to control spore-forming bacteria.

1.2 Project objectives

- To compare and screen the antibacterial and antioxidant activity of spice extracts *in vitro*.
- To determine the chemical composition (e.g. protein, lipid, carbohydrate,) and the shelf life (e.g. microbiological, chemical, physical and sensory evaluation test) of VP doner kebab stored at 4°C.
- To evaluate the effect of storage temperature, rosemary extracts, and packaging type on the stability losses and oxidation rate of high fat meat product (doner kebab) during an accelerated shelf life study.
- To assess the role of novel technology (vacuum packaging, modified atmosphere package, spice and herbs addition) to extend the storage shelf life of cooked doner kebab by measuring the sensory properties, Thiobarbituric acid reactive substances (TBARS) and microbiological levels.

- To evaluate the efficacy of spice extracts and MAP to fat *L. monocytogenes* inoculated in ready to eat doner kebab storage at 4°C in a challenge test protocol.
- To determine the process lethality and log reduction of spore forming bacteria in doner kebab and to predict the growth of *C. perfringens* in doner kebab roll during cooling time by using Pathogen Modelling Program / Cooling growth model.
- To apply knowledge on the effect of thermal inactivation and sumac addition on spore forming bacteria in RTE doner kebab.
- To evaluate the mathematical models as an adjunct for the determination of the growth, survival and death of pathogenic bacteria under diverse environmental conditions.

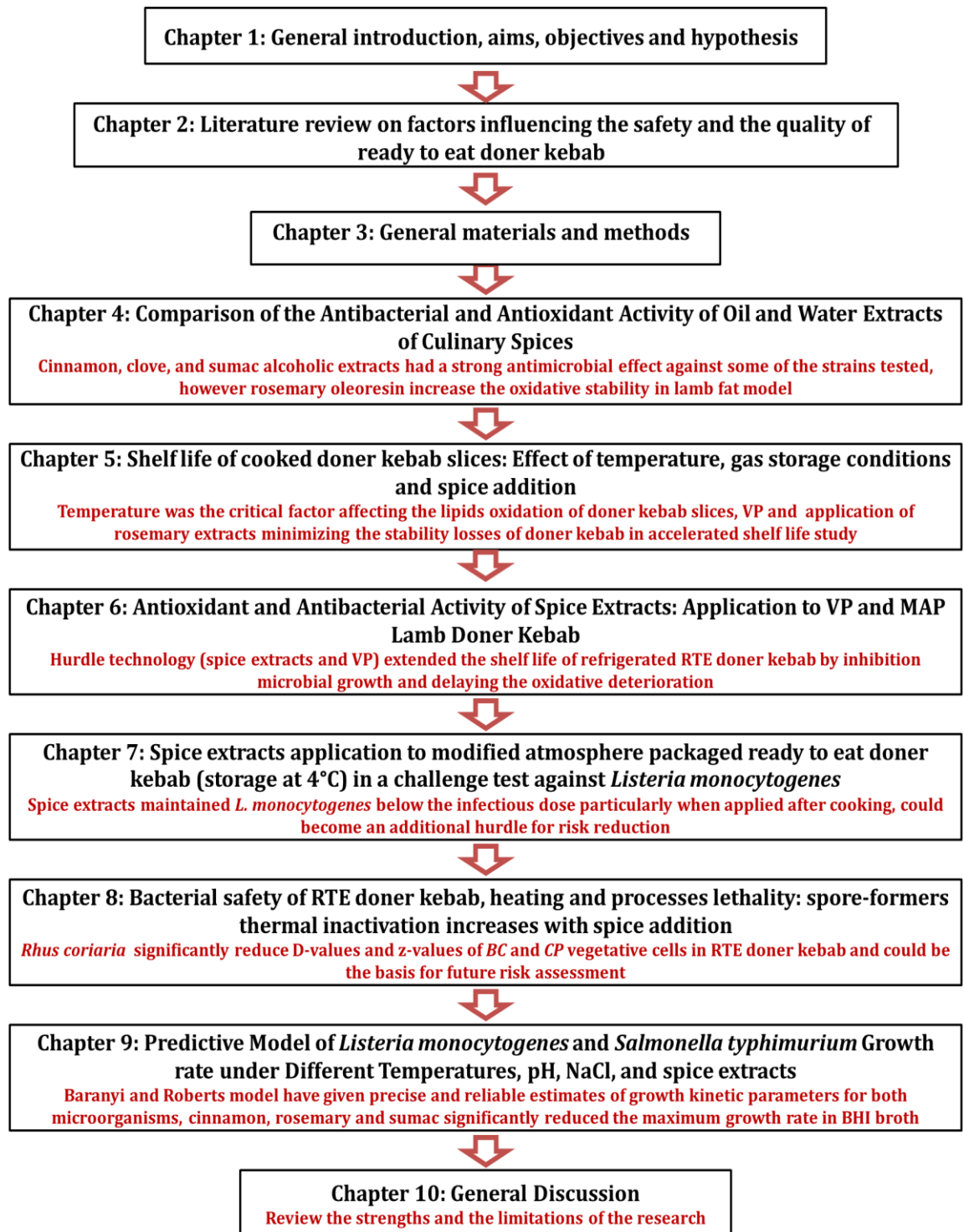


Figure 1.1: A schematic outline of the thesis with the main outcomes.

Chapter 2

2 Literature Review on factors influencing the safety and the quality of ready to eat doner kebab

2.1 Kebab products role in food-borne disease, their composition and nutritional value

Kebab is a traditional Middle East meat product, which is consumed widely in many areas of the world (Bryan et al. 1980, Bartholoma et al. 1997, Kilic 2003). The kebab is also known by some other names such as gyro, donna-kebab, doner kebab, chawarma or shawirma (Todd et al. 1986). Recently, consumption of processed meat product sold from fast food restaurants has been gaining popularity in many development countries including UK (Todd et al. 1986, Gordon et al. 1995). According to a UK survey of adults in 2002 there was a 10% increase in the buying of take-away food from doner kebab outlets between 1999 and 2002 (Mintel Market Intelligence 2002).

Doner kebabs usually consist of minced meat, fat or thin slices of lamb, which are seasoned with onion, pepper, tomatoes, and some spice. A core shape is given to the mix that is refrigerated to allow the meat and fat particles to stick together. Raw doner kebab is placed on a special doner kebab spit, slowly rotated in front of a heating element to roast the surface evenly, and then cut into thin slices using very sharp knives. The kebab slices are served either on a plate or in bread with additional herbs, salads or dressing (Todd et al. 1986, Kilic 2003). In recent year's doner kebab products, after preparation have been packaged in a plastic tray and held in refrigerated condition in supermarket for retail (Kayisoglu et al. 2003, Gonulalan et al. 2004).

A ready to eat doner kebab study was the third in the national co-ordination food liaison group sampling programme for 1995/1996 in UK (Gordon et al. 1995). A Local Authorities Co-ordinated Body on Food and Trading Standards and the Public Health Laboratory Service (LACOTS/PHLS) survey of the microbiological quality of doner kebabs in restaurants, takeaways and other retail premises in England and Wales was carried out in 1995 to identify the hygienic problems associated with this type of meat products at point of sale. According to the PHLS microbiological guidelines for ready-to-eat foods at the point of sale (Table 2.1), doner kebab was considered as safe to eat, although 5/2538 samples (0.2%) were unacceptable (potentially hazardous) and 307 (12%) were unsatisfactory (Gordon et al. 1995). The unacceptable samples involved three samples with high counts of *Clostridium perfringens*, one with *Salmonella* Mbandaka, and one with a high count of *Staphylococcus aureus*, whereas, the unsatisfactory samples had high aerobic plate counts. This survey indicated that doner kebab is generally safe to eat but that occasional infections could occur because of cross contamination.

Furthermore, a later survey, was performed in 2001, by the Greater Manchester/ Lancashire/PHLS food liaison group on 289 doner kebab samples from take-away food outlets in the region (Williamson et al. 2001). The samples consisted of pitta bread filled with doner kebab meat, salad, and sauce or yoghurt dressing. According to PHLS guidelines (Table 2.1), 4% of doner kebab samples were unsatisfactory / unacceptable due to the levels of *Bacillus subtilis* and *Staphylococcus aureus*. Follow up work showed that doner kebabs served with chilli sauce covered 11/12 of the unsatisfactory/unacceptable results.

In 2004, the data reported by the Health Protection Agency on the microbiological status of doner kebabs pointed out that 80% of the 397 doner

kebab samples tested were satisfactory, 16% were acceptable, 4% were unsatisfactory and none of the samples were of unacceptable microbiological quality (Burgess and Little 2004).

The cases reported by the Health Protection Agency's Communicable Surveillance Centre of Infectious Intestinal Disease related with doner kebab restaurants and/or kebabs, in England and Wales from 1992-2003 are illustrated in Table 2.2.

The microbiological quality of doner kebab depends on several factors such as the quality of raw materials, efficiency of the cooking process, sanitation of the kebab making facility and personal hygiene (Todd et al. 1986, Gould 1996, Roberts 2000, Politeo et al. 2006, Zeynep and Perihan 2006).

In Turkey, Kayisoglu et al. (2003), reported that raw and cooked beef and chicken kebab are contaminated with *Salmonella spp.*, *Clostridium perfringens*, psychrotrophic bacteria and coliforms, which indicates that doner kebab in the Turkish market could have very low hygienic quality. Therefore, they have a potential public health issue in this region.

Furthermore, Vazgecer et al. (2004), made similar observations on chicken doner samples, they reported that cooked doner kebab was a potential risk in Turkish restaurants especially when chicken skin was added, they also reported that food poisoning may occur when no proper time–temperature conditions during heating, cooling and storage condition of doner kebab were used.

Gonulalan et al. (2004), stated that raw and cooked doner kebab made from beef and sucuk dough (traditional fermented sausage) had a higher aerobic plate count (APC), psychrotrophic bacteria and coliform count, and the effect of storage on the microbial count increased with time. Therefore, the initial

microbial counts of the doner (beef and sucuk dough) were higher when comparing between 30 and 60 days of storage in – 30°C, although the difference between both times was not significant. Additionally, in Australia, Pointon *et al.* (2006) reported that a great hazard from *Salmonella spp.* occurred in ready to eat doner kebab due to inadequate cooking processes.

Considering the microbiological status mentioned above, issues such as poor heat transfer in meat, specially fats, shape, size and cooking systems that allow variation in the temperature control of doner kebab, each contribute to potential food-borne disease (Todd et al. 1986, Gordon et al. 1995, Evans et al. 1999).

Table 2.1: PHLS (UK) Guidelines for the microbiological quality of ready -to- eat doner kebab.

| Criterion | Microbiological quality (CFU/ g; or detected in 25g) | | | |
|---|--|---|-----------------------------------|---|
| | Satisfactory | Borderline - limit of acceptability | Unsatisfactory | Unacceptable/ potentially hazardous |
| Aerobic colony count | <10 ⁴ | 10 ⁴ -<10 ⁵ | ≥10 ⁵ | N/A |
| Indicator organisms | | | | |
| - Enterobacteriaceae | <100 | 100-<10 ⁴ | ≥10 ⁴ | N/A |
| - <i>E. coli</i> (total) | <20 | 20-<100 | ≥100 | N/A |
| - <i>Listeria</i> spp (total) | <20 | 20-<100 | ≥100 | N/A |
| Pathogens | | | | |
| - <i>Salmonella</i> spp | n.d. | | | detected |
| - <i>Campylobacter</i> spp | n.d. | | | detected |
| - <i>E. coli</i> O157 | n.d. | | | detected |
| - <i>V. cholerae</i> | n.d. | | | detected |
| - <i>V. parahaemolyticus</i> | <20 | 20-<100 | 100-<10 ³ | ≥10 ³ |
| - <i>L. monocytogenes</i> | <20 | 20-<100 | N/A | ≥100 |
| - <i>S. aureus</i> | <20 | 20-<100 | 100-<10 ⁴ | ≥10 ⁴ |
| - <i>C. perfringens</i> | <20 | 20-<100 | 100-<10 ⁴ | ≥10 ⁴ |
| - <i>B. cereus</i> and other pathogenic <i>bacillus</i> spp. | <10 ³ | 10 ³ -<10 ⁴ | 10 ⁴ -<10 ⁵ | ≥10 ⁵ |

N/A – not applicable; n.d. – not detected in 25 g, Adapted from (Gilbert et al. 2000) .

Table 2.2: Outbreaks of Infectious Intestinal Disease linked with Kebab restaurants and/or kebabs in England and Wales (Advisory Committee on the Microbiological Safety of Food 2004).

| Year | Restaurant specifics | Pathogen/toxin | Affected | Hospital | Vehicles ¹ |
|------|----------------------|-----------------------------|----------|----------|--------------------------------|
| 1992 | Indian takeaway | <i>S. Enteritidis</i> PT4 | 11 | 1 | Chicken, doner kebab |
| 1992 | takeaway | <i>S. Mikawasima</i> | 11 | 1 | Doner kebab |
| 1993 | Greek takeaways | <i>S. Mikawasima</i> | 64 | 4 | Doner kebab |
| 1993 | Indian | <i>S. Typhimurium</i> DT104 | 7 | 1 | Onion, Kebab |
| 1999 | Turkish kebab shop | VTEC O157 PT2 | 12 | 2 | Chicken kebab |
| 1999 | Indian | <i>S. Enteritidis</i> PT4 | 3 | | Chicken tikka kebab |
| 1999 | Takeaway kebab house | <i>S. Hindmarsh</i> | 12 | 3 | Lamb doner kebab, Salad, sauce |
| 2002 | Take away | <i>Campylobacter spp.</i> | 3 | 0 | Chicken kebabs |
| 2002 | Take away kebab shop | <i>S. Typhimurium</i> DT104 | 5 | 3 | Chicken |
| 2003 | Take away kebab shop | <i>S. Enteritidis</i> PT56 | 340 | 65 | Miscellaneous foods |

¹More than one vehicle can be reported in any given outbreak.

On the other hand, The LACORS coordinated food standards sampling programme (2009) looked at the content, total composition and nutrition values of doner kebabs across the UK. The information provided in this programme was the result of work by 76 individual councils collecting 494 samples of doner kebabs. That affords valuable information for consumers about the doner kebabs that they eat (Table 2. 3).

The data obtained from LACORS (2009) indicated that, according to the traffic light system, 97% of doner kebabs would be red for fat, 98% of the kebabs would be red for saturates and 96% of the kebabs would be red for salt. Thus, doner kebab should be reformulated, consumed in moderation or avoided.

Table 2.3: Mean and range of nutrient value and energy for different sizes of doner kebab samples and the guideline daily allowance according to (Committee on Medical Aspects of Food and Nutrition Policy 1991).

| Kebab Size | Total fat (g) | Saturate (g) | Carbohydrate (g) | Protein (g) | Salt (g) | Calories kcal |
|------------------|------------------|-------------------|-------------------|-------------------|-------------------|------------------|
| Large | 76.5 27.6-130 | 36.5 11.1-69.2 | 57.9 33.3-88.1 | 68.1 25.4-124 | 6.6 1.87-11.4 | 1193 525-1853 |
| Medium | 61.4 25.2-126 | 28.0 8.1-58.9 | 51.7 27.3-82.4 | 56.9 26.3-91.1 | 5.85 2.23-12.4 | 986 495-1725 |
| Small | 59.8 6.7-122 | 28.4 1.1-63.3 | 52.2 21-136 | 54.8 23.0-112 | 5.6 0.47-15.3 | 967 396-1742 |
| F/M ¹ | 70/95g | 20/30g | 230/300g | 45/55g | 6.0g | 2000/2500 |

¹: Female/Male

2.2 Food-borne bacteria and their relevance in food safety

Bacteria serve a critical role in meat products; they are responsible for most food poisoning and food-borne infection in kebab.

There are two categories of food-borne bacteria:

- Intoxication occurs only when large number of bacteria or large quantity of bacterial toxin has been ingested (Murray et al. 1995, Engel et al. 2001).
- Food-borne infection caused by relatively few organisms colonise in the body of victim over time (Engel et al. 2001).

2.3 Important food-borne bacteria in kebab

2.3.1 *Clostridium botulinum*

The species *Clostridium botulinum* includes a group of Gram positive, spore forming, anaerobes that form a powerful neurotoxin. There are seven types of botulinum toxin, but only types A, B, E and F cause illness in humans (Smith 2005). Food-borne botulism is caused by proteolytic *C. botulinum* and with non-proteolytic *C. botulinum*. Both types are physiologically and genetically distinct organisms. Proteolytic *C. botulinum* is a mesophile that produces heat resistant spores, whereas non-proteolytic *C. botulinum* is psychrotrophic and produce moderate heat resistant spores (Table 2.4).

Table 2.4: Characteristics of the two types of clostridia most frequently associated with food-borne botulism (Peck et al. 2006).

| Characters | <i>Clostridium botulinum</i> | |
|---|------------------------------|-----------------|
| | Proteolytic | Non-proteolytic |
| neurotoxins formed | A, B, F | B, E, F |
| minimum growth pH | 4.6 | 5.0 |
| minimum growth temperature | 10-12°C | 3.0°C |
| maximum growth NaCl | 10% | 5% |
| spore heat resistance (D _{100°C}) | 15 min | <0.1 min |

Food-borne botulism is a rare disease but its manifestations can be severe and can progress rapidly (Rhodehamel et al. 1992). It is an intoxication, resulting from consumption of foods in which *C. botulinum* has grown and produced botulinum toxin. Smith (2005), reported that as little as 30 ng of neurotoxin is sufficient to cause illness and even death. According to Lund and Peck (2000), the consumption of 0.1g of food in which *C. botulinum* has grown can result in botulism. The symptoms associated with botulinum appear 18 to 36h after consumption of contaminated food, and they may be gastrointestinal especially in type E with nausea, vomiting, abdominal pain or diarrhoea. Constipation will predominate after the onset of neurological symptoms, which include dizziness, difficulty in swallowing, diplopia, ophthalmoplegia, dysphagia and weakness in limbs (Shapiro et al. 1998). Outbreaks of food-borne botulism have been associated with foods sealed in airtight containers such as VP and MAP foods; however, the presence of air cannot be relied upon to prevent growth and toxin formation by *C. botulinum*. Such foods can contain oxygen free areas that will allow *C. botulinum* to grow and produce toxin (Food Standards Agency 2008).

In view of the severity of botulism, many guidelines and strategies were applied to control the growth of *C. botulinum* in foods and specifically in VP and MAP chilled foods (Peck et al. 2006). Preservation techniques such as high temperature, acidification, dehydration, curing (NO₂) and sanitation could be useful for inhibiting *C. botulinum* growth in VP and MAP chilled foods (Ranken 2000, Food Standards Agency 2008).

2.3.2 *Clostridium perfringens*

Clostridium perfringens is Gram positive, anaerobic, mesophilic, spore forming bacteria that is widely distributed in the environment and frequently occurs in the intestines of humans and many domestic and wild animals as a member of the normal flora (Saito and Funabashi 1991). *C. perfringens* strains are classified in to five groups (types A-E), type A is normally associated with food-borne illness, which ranks as the third most common food-borne disease (McClane 2007). The illness occurs when large number of bacteria sporulate in the intestine and produce *C. perfringens* enterotoxin (Barbara et al. 1999). The common form of *C. perfringens* food poisoning is characterized by intense abdominal cramping, nausea, and acute diarrhoea and usually occurs 8-24h after consumption of foods containing large numbers of *C. perfringens*, which are capable of producing the food poisoning toxin. The illness usually lasts about 24h. A mortalities have been reported due to dehydration or other complications (Engel et al. 2001, Vazgecer et al. 2004).

Outbreaks of *C. perfringens* are commonly associated with cooked food in which large quantities are prepared and held for long time at ambient temperature or cooled too slowly after cooking (Bean and Griffin 1990, Vazgecer et al. 2004). One type of food of particular relevance is large bulk meat products such as doner kebab (Gordon et al. 1995, Peck et al. 2004, Atwa and Abou El-Roos 2011).

The spores of *C. perfringens* isolated from food poisoning outbreaks exhibit high resistance to various environmental stresses such as: prolonged frozen stage, moist heat and high hydrostatic pressure (Paredes-Sabja et al. 2007, Perez-Lamela and Torres 2008).

The temperature range for growth of *C. perfringens* is 15– 52°C, with very rapid growth at temperatures between 43°C and 47°C (doubling times of less than 10 min have been reported in meat) (Willardsen et al. 1979). Additionally, slow cooling may allow germination of spores that have survived cooking, leading to rapid multiplication of the organism to an infectious dose. The application of appropriate effective cooling regimes to foods should substantially reduce the incidence of food-borne illness associated with *C. perfringens* (Peck et al. 2004). Control measures emphasize proper food preparation, storage and temperature controls to prevent *C. perfringens* food poisoning outbreaks. These include: rapid uniform cooling of cooked foods, hot holding of cooked foods and reheating cooled or chilled foods to a minimum internal temperature of 74°C (Food Safety and Inspection Service and United States Department of Agriculture 2003).

2.3.3 *Staphylococcus aureus*

Staphylococcus aureus is one of the pathogens that cause acute bacterial food poisoning and over half of documented food poisoning cases are caused by this bacterium. It is Gram-positive coccus non-motile, non-sporing and facultative anaerobe (International Commission on Microbiological Specifications for Foods 2005). *S. aureus* produce enterotoxins during all phases of growth, particularly during the middle and at the end of the exponential phase and causes illness when the preformed toxin is ingested in food. The illness and toxin production occur when *S. aureus* grows to approximately 10^5 CFUml⁻¹ or more (Bergdoll 1989). The principal symptom of staphylococcal food poisoning is vomiting within 6 h after eating contaminated food and usually followed by abdominal pain, and diarrhoea (Phillips 1998).

Many studies have reported the presence of *S. aureus* in foods (Balaban and Rasooly 2000, Andrea et al. 2011, Hennekinne et al. 2011). In a study of food poisoning in England, meat, poultry or their products were the most prevalent contaminated foods (75%) (Wieneke et al. 1993). Other contaminated food products included fish and shell-fish (7%) and milk products (8%) (Wieneke et al. 1993). The nutritional requirements of *S. aureus* are complex and vary from strain to strain. The conditions under which this bacterium grows also depend on the composition of the food. In general, *S. aureus* grows between 7°C and 47°C, with an optimal temperature of 30°C to 37°C. Enterotoxins are produced between 10°C and 46°C, with an optimum temperature of 35-45°C. Enterotoxin production is considerably reduced at 20-25°C. In general, the enterotoxin production is unlikely to occur at temperatures below 10°C (Lund et al. 2000). Optimal enterotoxin production occurs at pH 6-7 and it is affected by atmospheric conditions and salt level (Miller et al. 1997, Lund et al. 2000).

2.3.4 *Campylobacter* spp.

Campylobacter infections are among the most frequent cause of bacterial enteric infection in humans (Solomon and Hoover 1999, Parkhill et al. 2000, Bang et al. 2001, Snelling et al. 2005, Humphrey et al. 2007). They produce both diarrhoeal and systemic illnesses. In industrialized regions, enteric *Campylobacter* infections produce an inflammatory, sometimes bloody, diarrhoea or dysentery syndrome (Phillips 1998).

Campylobacter spp. are widespread in the intestinal tract of warm-blooded animals used for food production. They may therefore readily contaminate raw meat, raw milk and raw milk products. *Campylobacter* spp. are also commonly found in the intestine of healthy birds and most raw poultry meat has

campylobacter on it (Food Safety Authority of Ireland 2002). This relates to the high optimum growth temperature (42°C) of the microorganism, which approximates the normal body temperature of poultry (Food Safety Authority of Ireland 2002). Transmission of *Campylobacter* organisms to humans usually occurs via infected animals and their food products. Most human infections result from the consumption of improperly cooked or contaminated foodstuffs. Chickens may account for 50-70% of human *Campylobacter* infections. (Kapperud et al. 2003). The most important species responsible for food-borne infections in humans are *Campylobacter jejuni* and *Campylobacter coli*, which are both sensitive to stress conditions (Humphrey et al. 2007). *Campylobacter* spp. are Gram negative, spiral, fragile organisms. They are sensitive to heating (pasteurisation/cooking), freezing (Chan et al. 2001, Zhao et al. 2003), drying, acidic conditions (pickling), disinfectants and irradiation. They survive poorly at room temperature (21°C) and in general survive better at refrigeration temperatures. They can grow on moist foods at temperatures ranging between 30°C and 45°C, with an optimum temperature of 42°C (Food Safety Authority of Ireland 2002). Food containing low number of *Campylobacter* spp., ranging from 500 to 10,000 cells is sufficient to cause illness. Therefore, the fact that the organism cannot multiply very effectively in most foods does not prevent it from causing food-borne illness.

The *Campylobacter* spp. associated with human disease required specific atmospheric conditions, growing best in an atmosphere containing 10% carbon dioxide and 5-6% oxygen. In the presence of air the organisms die off quickly and the vacuum or gas packaging condition appears to have little effect on their survival (Engel et al. 2001).

2.3.5 *Salmonella* spp.

Salmonella species are one of the most common causes of food-borne illness in humans (Cardinale et al. 2005). Poultry is an important reservoir for salmonella and considered as a main source of human salmonellosis (Wilson 2002, Capita et al. 2003, Capita et al. 2007).

They are Gram-negative, facultative anaerobic, non-spore forming short rods, which grow under a wide range of conditions, multiply in both aerobic and anaerobic conditions and within pH range from 4 to 8. Their optimum growth temperature is about 38°C - 41°C and their minimum is about 5°C (Forsythe 2010). *Salmonella* is resistant to freezing and drying but they are relatively heat sensitive, being killed at 60°C for 15-20 minutes and acid below pH4 (Forsythe 2010).

Salmonella can contaminate RTE products in the following ways (United States Department of Agriculture and Food Safety and Inspection Service 2011):

1. Under processing

Under processing occurs when the lethality treatment is not adequate to eliminate the *Salmonella* spp..

2. Cross contamination

- Contamination from ingredients added after the lethality treatment

Salmonella contamination may occur due to the addition of uncooked vegetables, fresh herbs, eggs, and spices to processed meat and poultry products after the primary lethality treatment. Thus, the safety of all ingredients added to the product after cooking must be considered.

- Raw meat and poultry may contaminate finished products by direct or indirect routes such as contaminated equipment surfaces, environmental sources, food handlers

- Contamination from food handlers
- Contamination from insect or animal vectors

2.3.6 *Listeria monocytogenes*

Food-borne *L. monocytogenes* is a serious threat to human health (Guenther et al. 2009). *L. monocytogenes* is an opportunistic pathogen, which is commonly present in the environment and can be isolated from meat and meat products, particularly ready to eat meat product (RTE) (Uyttendaele et al. 1999, Uyttendaele et al. 2004, Mejlholm et al. 2010).

It is a Gram positive bacterium, facultative anaerobic, psychrotrophic and can grow and multiply in a wide range of conditions (Russell et al. 1999) (Table 2. 4). This poses a special problem for food handling, storage and food processing surfaces especially in ready to eat foods (Engel et al. 2001, Guenther et al. 2009).

Table 2.5: Growth and Survival Limits for *L. monocytogenes* in food (Food Safety Authority of Ireland 2005).

| Parameter | Minimum | Maximum | Optimal | Can survive (but no growth) ² |
|----------------------------------|-------------|-----------|---------|---|
| Temperature (°C) | -1.5 - +3 | 45 | 30 -37 | -18°C |
| pH ¹ | 4.2 - 4.3 | 9.4 - 9.5 | 7.0 | 3.3 to 4.2 |
| Water Activity (w _a) | 0.90 - 0.93 | > 0.99 | 0.97 | < 0.90 |
| Salt (%) | < 0.5 | 12 to 16 | N/A | ≥ 20 |

¹: Hydrochloric acid as acidulant (inhibition is dependent on type of acid present),

²: Survival period will vary depending on nature of food and other factors, N/A Not Applicable.

L. monocytogenes is a food-borne pathogen responsible for high mortality in infected persons from at risk groups (e.g. new-borns, elderly and pregnant women). According to Food Standards Agency(FSA) (2007b), *L. monocytogenes* caused 37% of foodborne mortality in England and Wales (see Figure 2.1).

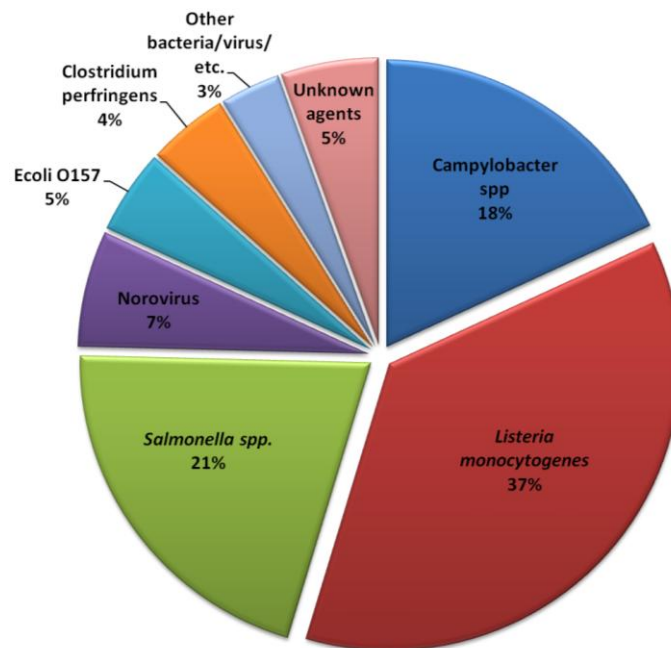


Figure 2.1: Estimated death cases (mortality) in England and Wales due to Food-borne infections, Food Standards Agency 2007.

2.3.7 *Escherichia coli*

Escherichia coli is a normal inhabitant of healthy human and animal intestines, most strains are harmless, but certain strains are pathogenic (Padhye and Doyol 1992, Engel et al. 2001). The pathogenic strain is *E. coli* O175:H7 which is recognized worldwide as a food-borne pathogen especially in children and elderly (Hussein 2007, Sharma et al. 2008). *E. coli* O157:H7 are Gram-negative rods that have been variously described as verotoxigenic *E. coli* (VTEC) or shiga-like toxin producing *E. coli* (SLTEC) (Gyles 1992). These potent toxins are the cause of severe damage to the intestinal lining of those infected. The infection can lead to severe symptoms in humans including haemorrhagic colitis, haemolytic uremic syndrome or thrombocytopenic purpura and in some cases death, making this pathogen a significant public health concern (Banatvala et al. 2001, Willshaw et al. 2001, Messelhausser et al. 2007). *E. coli* can survive at low temperatures and under acidic conditions, making it difficult to eradicate in nature. The organism has a low infective dose and can be transmitted from person to person. Sources of *E. coli* O157:H7 infections include undercooked or raw meat and poultry products, unpasteurized fruit juices, dry-cured salami, lettuce, cheese curds, unpasteurized or raw milk, contaminated water and ice, and person-to-person transmission. Meat is still recognized as a primary vehicle of infection and a large numbers of out breaks have been linked to consumption of contaminated and improperly cooked minced beef (Koohmaraie et al. 2005). Since there appears to be a very low infective dose for this organism (10–100 cells), adequate sanitation and proper processing of foods is critically important.

2.4 Rancidity and meat deterioration

Rancidity is one of the primary mechanisms of quality deterioration in meat and meat products, which occurs due to either lipid oxidation or hydrolytic activity. Additionally, it could seriously impair food desirability by changing the flavour, odours and colour (Baardseth et al. 2005). Rancidity is not only considered as a quality problem but it also has an effect on health and disease (Kenji and Mitsuo 1998). There are two types of rancidity (Ranked 1983):

2.4.1 Oxidative Rancidity (lipid oxidation of meat and meat products)

Lipid oxidation is a common process of meat and meat product deterioration which occurs when meat lipids oxidize and interact with other meat constituents, such as pigments and other proteins, carbohydrates and vitamins leading to undesirable changes in flavour texture, colour and nutritive value (Fernández et al. 1997a, Baardseth et al. 2005).

Lipids in skeletal muscle formed about 1-13% of total muscle. Main lipids are found intramuscularly, intermuscularly and in adipose tissue. Intramuscular lipids are mainly composed of triglyceride, stored in fat cell, and phospholipids, located in cell membranes. Intermuscular and adipose tissue lipids are mainly composed of triglycerides. Lipid in the muscle can be classified into two groups:

- Triglycerides. These are the major constituents of fat.
- Phospholipids. These are present in minor amounts but have a strong importance for flavour development and oxidation in meat and meat products (Fidel 2002).

The phospholipids was have a relatively higher proportion of the polyunsaturated fatty acids than triglycerides, thus phospholipids are prone to oxidation process very quickly (Ranked 1983).

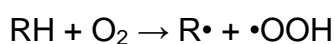
The amount of total lipid and neutral lipid has been shown to be variable in muscles from different animals, whereas phospholipid is relatively constant (Love and Pearson 1971). Tichivangana and Morrissey (1985) reported that the fatty acid composition of phospholipids shows a notable variation within animal species, and the auto-oxidation of muscle foods occurs in the following descending order: fish, poultry, pork, beef and lamb. Furthermore, the compositional differences in fatty acids in the phospholipid fraction may result in the variation susceptibility to oxidative rancidity, fatty acids namely oleic, linoleic and linolenic acids were found to produce low molecular weight aldehydes which are responsible for the development of warm-over flavour (WOF) and rancidity in storage meat products (Leo et al. 2007).

Many factors seem to affect lipid oxidation in animal tissues including: species, sex, age, anatomical location, diet, environmental temperature, light, exposure to air, and phospholipid composition and content. In addition, processing, handling and storage conditions of meat and meat products also have a potential effect on lipid oxidation (Ladikos and Lougovois 1990).

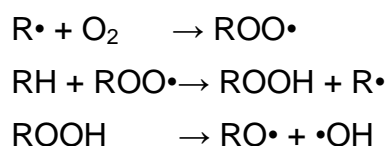
Lipid oxidation is a complex process commonly described as an oxidative, oxygen dependent, deterioration of fats, notably the unsaturated fatty acids.

This modification of fatty acids is principally carried out by an autocatalytic mechanism of 'free radicals', called auto oxidation, consisting of three stages (Ranked 1983, Raharjo and Sofos 1993, Leo et al. 2007).

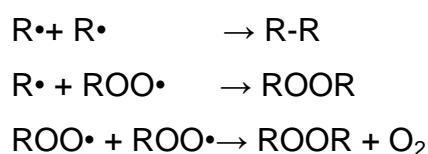
Initiation stage (free radical formation): In this stage a small number of highly reactive, short life fatty acid molecules arise. These are the free radicals (R.):



Propagation stage (free radical chain reaction): The free radicals react with atmospheric oxygen to generate proxy radicals, which are also highly reactive, and act with other unsaturated fatty acid to produce hydro peroxides (ROOH), and other free radicals that are hydrolysed to a proxy radical and free radical:



Termination stage (formation of non-radical products): free radicals combine to give stable end products.



Lipids hydroperoxides (ROOH) are the primary lipid oxidation products; they are non-volatile and odorless, but relatively unstable compounds (labile species, of very transitory nature), which undergo changes and deterioration with the radicals. Their breakage produces volatile and non-volatile secondary products such as aldehydes, alcohols, ketones, and various carbonyl compounds (Pearson et al. 1983, Raharjo and Sofos 1993, Fernández et al. 1997a). Many studies have been reported their effect on develop off-odour and off-flavour in meat product (Vercellotti et al. 1992, Byrne et al. 2001).

Kenji and Mitsuo (1998), reported that secondary products of lipid oxidation particularly malondialdehyde (MDA) is thought to be a carcinogenic initiator and mutagen, and thus can affect the safety of food.

2.4.2 *Hydrolytic rancidity*

Hydrolytic rancidity occurs in meat and meat products under certain circumstances. The hydrolytic rancidity in meat may be as a result of direct chemical causes (acidity) which is very rare in meat and meat product, or due to enzyme activity (Ranked 1983).

Hydrolytic changes initiated by enzyme activity may occur in meat due to microbiological growth. These micro-organisms induced lipolytic and proteolytic changes. The lipolytic enzyme produced by microorganism on the meat surface, particularly by mould and sometimes by yeast or bacteria, which produce rancidity on the surface fat (Lawrie 1985). However, meat is considered spoiled when the general level of microbiological activity usually leads to such obvious signs as slime, mouldiness or smell (Ranked 1983).

2.5 Colour deterioration

Meat colour is considered an important factor to determine the shelf-life of meat products because customers tend to use meat colour as the main indication of freshness and wholesomeness. The colour of meat is influenced by several factors such as the concentration of pigments particularly myoglobin in muscle tissue, the chemical status of these pigment and the physical characteristic of meat (Decker et al. 2000).

Muscle tissue contains different amounts of pigments including myoglobin, haemoglobin, and cytochrome C (Mancini and Hunt 2005). Myoglobin is the primary pigment (80%-90%) responsible for meat colour (Vaclavik and Christian 2007), however, meat colour is affected by haemoglobin and also cytochrome C. Myoglobin is the protein within the muscle fibre, which carries oxygen from capillaries to the intracellular locations of muscle tissue and facilitates muscle

movement (Renner 2000). Haemoglobin is the protein that carries oxygen from the lungs to the muscle cell or fibre and cytochrome c is a protein associated with mitochondria and is involved in electron transport (Claus 2007).

Myoglobin is a water-soluble protein, consists of a protein portion that is known as globin and a non-protein portion, which is a haem ring (e.g. protoporphyrin). The globular protein consists of 153 amino acids that are 80% in the alpha-helix conformation with the interior of the protein consist of mostly non-polar residues. In contrast most of the polar residues are located at the myoglobin surface, and the only polar residues that are located in the core of the protein are the two histidines that have a critical function at the haem binding site (Decker et al. 2000). The heme ring has a centrally located iron atom that can form six bonds. Four of these bonds are with pyrrole nitrogens while the fifth bond links the iron atom to the proximal histidine-93 residue of globin and a sixth site is available to reversibly bind a variety of ligands (Decker et al. 2000, Mancini and Hunt 2005). Both the valence of the iron and the ligand present dictate the meat and meat product colour (Figure 2.2). Three major chemical forms are principally responsible for meat colour; deoxymyoglobin, oxymyoglobin, and metmyoglobin.

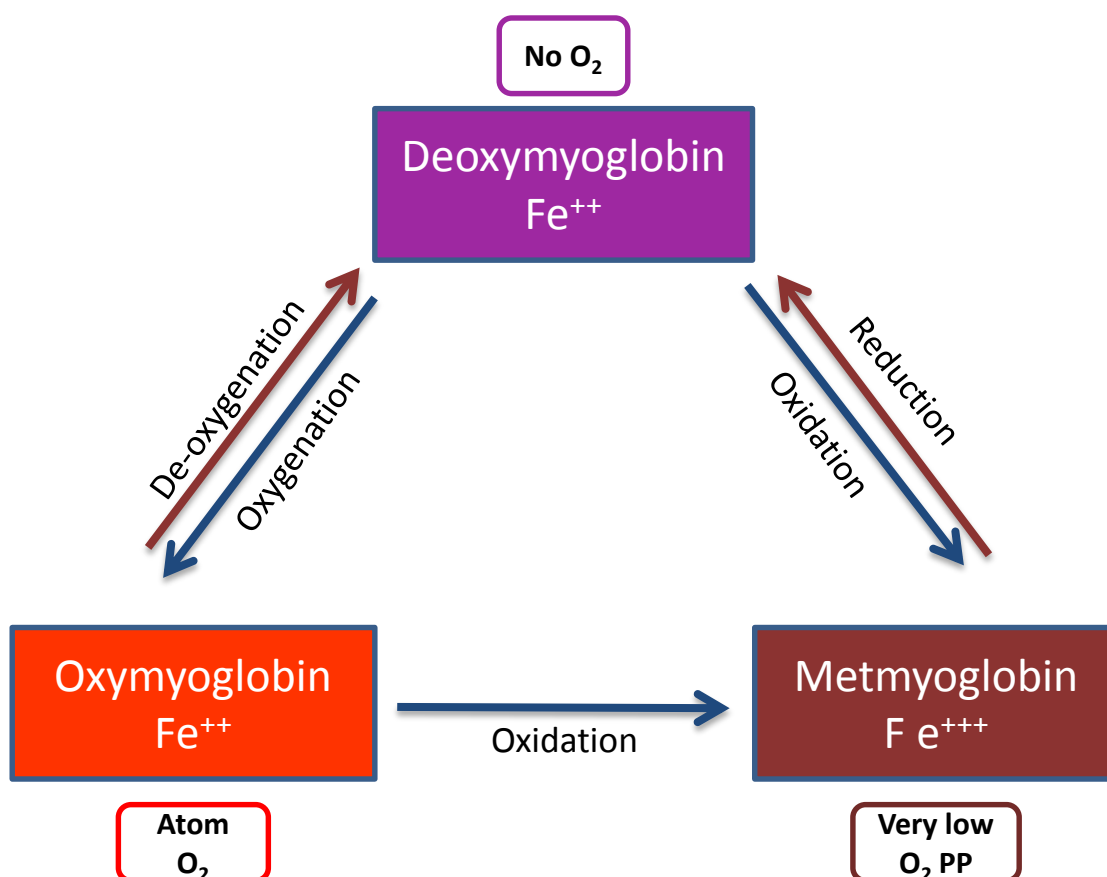


Figure 2.2: Myoglobin redox-forms.

2.5.1 Redox reaction between myoglobin forms

2.5.1.1 Oxygenation

Deoxymyoglobin characterized by purplish-red or purplish-pink colour occurs when no ligand is present at the 6th coordination site of heme iron (empty) and the heme iron is ferrous (Fe⁺⁺). This form is typically related with muscle immediately after cutting and vacuum packaged product (Mancini and Hunt 2005). To keep myoglobin in a deoxygenated form, very low oxygen tension is required (Brooks 1935). Oxygenation arises when myoglobin is exposed to oxygen and the result is a bright cherry-red colour. It is important to remember that during oxygenation the iron remains in the ferrous form (Fe⁺⁺) while the 6th coordination site is now occupied by diatomic oxygen. The sixth coordination site is not exclusively for oxygen; however, it can also bind with nitric oxide and

carbon monoxide in order to make a stable myoglobin complex that is important for meat colour (Renerre 2000).

2.5.1.2 Oxidation

Meat discoloration results from oxidation of both ferrous myoglobin derivatives (deoxymyoglobin and oxymyoglobin) to yield the brown or sometimes grey coloured metmyoglobin (ferric iron). The metmyoglobin (Fe^{+++}), cannot bind oxygen in its sixth coordination; instead, it binds water (Mancini and Hunt 2005). Discoloration is often indicated to as the amount of surface area covered by metmyoglobin; nevertheless, subsurface myoglobin forms also influence product appearance. This is due to the location of metmyoglobin between superficial oxymyoglobin and interior deoxymyoglobin that gradually thickens and moves towards the surface (Mancini and Hunt 2005).

Several factors could influence metmyoglobin formation such as oxygen partial pressure, temperature, pH, meat reducing activity, as well as microbial growth (Decker et al. 2000).

2.5.1.3 Reduction

Critical in meat colour life is a reduction of metmyoglobin, which is mainly dependent on metmyoglobin-reducing activity/system. Reduction process the brownish metmyoglobin is converted to the oxygenated red pigment. This reduction depends on the oxygen-scavenging enzymes, reducing enzyme systems and the NADH pool of the muscle, which is depleted as time post mortem progresses (Mancini and Hunt 2005, Claus 2007).

2.6 Preservation technologies for meat and meat products

Meat is considered as an ideal environment for the growth and propagation of food-borne pathogens and meat spoilage bacteria. Thus, it is important that adequate preservation technologies are used to maintain both safety and good quality of meat products (Aymerich et al. 2008, Zhou et al. 2010). Most preservative techniques aim to control all the form of quality loss that may occur, however the overriding priority always is to minimize the occurrence and growth of microorganisms particularly that cause food poisoning (Nychas et al. 2003).

Many factors influence the shelf life and keeping quality of meat products, including storage temperature, atmospheric oxygen (O₂), moisture (dehydration), light and most importantly, microorganisms. All of these factors, either alone or in combination, can result in detrimental changes in the colour odour, texture and flavour of meat (Faustman and Cassens 1990).

Methods of meat preservation may be grouped into two broad categories based on: firstly preventing or slowing the microbial growth which include freezing, chilling, drying, curing, vacuum packing, modified atmosphere packing acidifying, fermenting and preservatives (Kotzekidou and Bloukas 1996, Metaxopoulos et al. 2002), and secondly those involving inactivating of the microorganisms, such as pasteurization and sterilization (Gould 2000) (Table 2.6).

Table 2.6: Major preservation techniques for meats and meat products.

| Principle | Technique | Example |
|--|-------------------------------|---|
| Prevention the growth of micro-organisms | Reduction in temperature | chill storage, frozen storage |
| | Reduction in water activity | drying, curing with add salt |
| | Reduction in pH | Acidification (use acetic acid, citric acid) |
| | Removal of oxygen | vacuum or atmosphere packaging |
| | Modified atmosphere packaging | replacement of air CO ₂ , O ₂ , N ₂ mixtures |
| | Addition of preservatives | -inorganic (sulphite, nitrite) -organic (propionate, sorbet, benzoate) -bacteriocin -antimycotic (natamycin) |
| | Control of microstructure | in water in oil emulsion meat |
| Inactivation of micro-organisms | Heating | -pasteurization -sterilization |

Adapted from (Gould 1989).

Lawrie and Ledward (2006), reported that a combination of preservative techniques (hurdle technology) can be devised to reach particular objectives in terms of both microbial and organoleptic quality.

New preservation technologies attempt to be mild, environmentally friendly and guarantee natural appearance of meat and also to reach consumers demand towards nutritional and healthier food; high hydrostatic pressure (HHP), natural antimicrobial compounds and biopreservation (Table 2.7).

Table 2.7: New and emerging technologies for food preservation.

| New technologies for food preservation | |
|--|--|
| <u>Natural additives</u> | <u>Physical processing</u> |
| <ul style="list-style-type: none"> • Animal derived antimicrobial • Plant derived antimicrobial • Microbial product | <ul style="list-style-type: none"> • Gama and electron beam irradiation • High voltage electric gradient pulses • High hydrostatic pressure |

Sources (Gould 1995, Holzapfel et al. 1995, Arora and Kaur 1999, Zhou et al. 2010).

2.7 Spices and herbs as meat preservatives

Food contamination and deterioration are still of concern for both consumer and the food industry, although different preservation methods are used; consumers demand healthy food free from synthetic preservatives (Chastain et al. 1982, Chen et al. 1984). Therefore, the search for effective, nontoxic, and natural sources of antimicrobial and antioxidant compounds such as spices and herbs have notably increased in recent years (Loliger 1991, Ceylan et al. 2004, Ak and Gülçin 2008).

Spices and herbs have been added to foods since ancient times not only as flavouring agents but also as preservatives through their antioxidant and antibacterial activity (Beuchat and Golden 1989, Fernández-López et al. 2003). The international standard organization has defined the spices as vegetable products or mixtures thereof, used for flavouring and seasoning in food (International Organization for Standardization 1995). In Turkish food, spice is defined as a natural compound or mixture of natural compound that is extracted from the seeds, fruit, flower, or trunks of several plants and added to food in order to provide colour, taste, smell, or flavour (Agaoglu et al. 2007).

Previous studies demonstrated that the inhibitory effects of spices and herbs are mostly due to the volatile oils present in their composition, which have strong influences against several types of pathogens, such as compounds present in oregano, clove, cinnamon, garlic, mint, rosemary and cardamom (Angioni et al. 2004, Arqués et al. 2008). However, less antibacterial efficacy was obtained from black pepper, red pepper, chilli powder, cumin and curry powder (Holley and Patel 2005).

The antimicrobial efficacy of plant extracts is type and concentration dependent. As reported by Tajkarimi et al (2010), spices and herbs are mostly used in the

range of 0.05–0.1% in food systems. Nevertheless, some spices require higher concentrations. Shan et al. (2007b) demonstrated that many types of spice extracts contained high level of phenolic compounds that possessed strong antibacterial activity against some food-borne pathogen.

In meat products, previous studies have been shown that spice and herbs extracts can significantly reduce number of pathogenic and spoilage bacteria (Fernández-López et al. 2005, Karabagias et al. 2011). However, another study has reported low antimicrobial effects against pathogens in contaminated meat products (Tajkarimi et al. 2010). The effectiveness of spice extracts in meat products can be influenced by different factors such as: type and composition of the spice, amount used, type of microorganisms, composition of the food, pH value, temperature of the environment, protein, lipids, salts, and phenolic substances present in the food (International Commission on Microbiological Specifications for Foods 2005). Moreover, Burt (2004) and Lis-Balchin et al. (2003) indicated that high fat content in food has a negative effect on the application of plant essential oils (EOs). Eugenol and coriander, clove, oregano and thyme oils displayed high effects against *L. monocytogenes*, *Aeromonas hydrophila* and spoilage bacteria in meat products, whereas mustard, cilantro, mint and sage oils were less effective or ineffective (Burt 2004) .

A study carried out by Yin and Chao (2008) demonstrated that roselle extract (*Hibiscus sabdariffa* L.) considerably inhibited the growth of both susceptible and antibiotic-resistant *Campylobacter* spp. in contaminated ground beef.

Clove and tea-tree oils controlled *Escherichia coli* O157:H7 on blanched spinach and minced cooked beef (Moreira et al. 2007).

As suggested by Carramiana et al. (2008), a combination of spice EOs (winter savory) with other preservation methods, for example reduced temperature,

high pressure, irradiation, or packaging under a modified atmosphere can be used to control the growth of pathogenic bacteria and improve the quality of minced pork (Zhang et al. 2009).

Additionally, strong antimicrobial activity was observed from individual extracts of clove, rosemary, cassia bark and liquorice; and even a synergistic effect was achieved from the mixture of rosemary and liquorice extracts against *L. monocytogenes*, *E. coli*, *Ps. fluorescens* and *Lactobacillus sake*. in modified atmosphere-packaged fresh pork and vacuum-packaged ham slices stored at 4°C (Zhang et al. 2009). Recent studies regarding spice extract antimicrobial activity in meat products are summarized in Table 2.8.

On the other hand, spice and herbs extracts demonstrated a strong antioxidant property that positively influences meat quality characteristics by preventing lipid and pigment oxidation thus appears to be an alternative to synthetic additives in the meat industry (Deligeorgis and Simitzis 2010).

The vast majority of lipid oxidation is represented by deterioration of flavour, colour, texture, and nutritional value of food (Kanner 1994a). Thus, natural plant material (e.g. herbs and spices) have been used as potential antioxidants to maintain the quality of meat products (Zhang et al. 2010) (Table 2. 9).

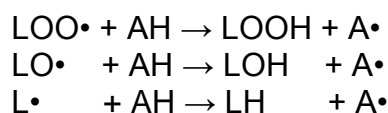
According to Shan et al. (2005), the phytochemicals compounds including phenolic diterpenes, flavonoids, tannins and phenolic acids that present in spice and herbs composition are the potential sources of natural antioxidants.

Frankel (1998) indicated that antioxidant components work by a variety of different mechanisms, including the control of both oxidation substance (e.g. oxygen and lipid) and prooxidants (e.g. reactive oxygen species and prooxidant metals) and the inactivation of free radicals.

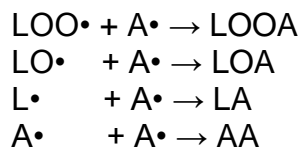
McClements (2005), reported that antioxidant compounds can be divided into two groups depending on the mechanism by which they operate:

- Primary antioxidants (chain-breaking antioxidant) retard lipid oxidation due to their ability to accept free radicals, hence either retarding the initiation step or interrupting the propagation step (McClement and Decker 2000, Chaiyasit et al. 2007).

The ability of antioxidant (AH) to react with lipid radicals (peroxyl radicals ($\text{LOO}\cdot$), alkoxyl radical ($\text{LO}\cdot$) and free radical ($\text{L}\cdot$)) as follows:



Antioxidant radicals ($\text{A}\cdot$) are also able to participate in termination reactions with other antioxidant radicals or lipid radicals to form non radical species as follows.



- Secondary antioxidant retard lipid oxidation through a range of mechanism such as chelating of transition metal, oxygen scavenging and deactivation of reactive species (Reische et al. 1998, Trojakova et al. 2001).

Table 2.8: List of studies reporting the antibacterial activity of spice extracts and their application to meat products.

| Meat product | Spice extracts | Concentration used | Target bacteria | Source |
|--------------------|--|------------------------|---|-------------------------------|
| Minced beef | Coriander and Hyssop | 0.02% v/w | <i>Enterobacteriaceae</i> | Michalczyk et al. 2012) |
| MAP and VP ham | Mixed rosemary and liquorice | 0.25% 0.5% 1% | <i>L. monocytogenes</i> | (Zhang et al. 2009) |
| Chicken sausage | Rosemary Chinese mahogany | 0.05%, 0.1%, 0.15% | TVC | (Liu et al. 2009) |
| Ground beef | Garlic, ginger, and turmeric | 5%, 7%, 5% | <i>S. Typhimurium</i> <i>DT104</i> | (Milagros et al. 2006) |
| Beef meatball | Rosemary oil extracts | 0.1% | LAB | (Fernández-López et al. 2005) |
| | Rosemary water extract | 0.15% | | |
| | Rosemary oil and water extracts | 0.25% | | |
| Mutton chunks | Clove | 2% | Lipolytic bacteria | (Kumudavally et al. 2005) |
| | Cinnamon | 2% | Enterobacteriaceae | |
| | Turmeric | 2% | | |
| Cooked ground beef | Chilli, Garlic, Curry, Clove and Oregano | 1% | <i>C. perfringens</i> spore | (Sabah et al. 2004) |
| Raw beef | <i>Capsicum annuum</i> bell pepper | 0.02%-2.5% 0.02%-5% | <i>S. Typhimurium</i> , <i>Ps. aeruginosa</i> | (Careaga et al. 2003) |
| VP ham | Cilantro oil | 0.1- 6% | <i>L. monocytogenes</i> | (Gill et al. 2002) |
| Ground beef | Ascorbic acid coated with a protein based cross linked film containing immobilized spice powders | 0.5% | Aerobic mesophilic microorganisms <i>B. thermosphacta</i> LAB | (Ouattara et al. 2002) |
| Minced beef | Oregano oil | 0.05% 0.5% 1% | Natural microflora | (Skandamis and Nychas 2001) |
| Minced mutton | Clove oil | 0.5-1% | <i>L. monocytogenes</i> | (Vrinda Menon and Garg 2001) |
| Beef fillets | Oregano oil | 0.8% | <i>L. monocytogenes</i> | (Tsigarida et al. 2000) |

Table 2.9: List of studies reporting the antioxidant activity of spice extracts (TBARS) and their application to meat products.

| Meat product | Spice extracts | Concentration Used | Source |
|---------------------------------------|---|--|------------------------------------|
| Mortadella-type Sausages | Winter Savory | 0.78% 1.56% 3.125% | (Coutinho de Oliveira et al. 2012) |
| Mortadella | Thyme Rosemary | 0.02% 0.02% | (Viuda-Martos et al. 2010a) |
| bologna sausages | Oregano | 0.02% | (Viuda-Martos et al. 2010b) |
| Raw pork batters | Rosemary and Oregano | 0.02% | (Hernández-Hernández et al. 2009) |
| Radiation processed lamb meat | Mint leaves | 0.1% | (Kanatt et al. 2007) |
| Cooked ground beef | Clove Star anise, Fennel, Pepper, and Cinnamon | 0.1% 0.5% 0.5% 0.5% | (Dwivedi et al. 2006) |
| Ground goat meat | White peony Red peony Sappanwood Moutan peony | 0.5–2.0% 0.5–2.0% 0.5–2.0% 0.5–2.0% | (Han and Rhee 2005) |
| Ground beef | Rosemary | 0.01–0.25% | |
| Beef meat balls | Citrus Rosemary oil Rosemary water Rosemary oil and water extracts | 5% 0.1% 0.15% 0.25% | (Fernández-López et al. 2005) |
| Cooked Pork Meat | Hyssopus and Rosemary Extracts | 11% | (Fernández-López et al. 2003) |
| Goat meat (sausage) | Rosemary | 0.05% | (Nassu et al. 2003) |
| Dry sausage* | Garlic and Paprika | 1%, 3% | (Aguirrezábal et al. 2000) |
| Raw and cooked minced beef | Galangal (Alpinia galanga) | 5-10% | (Poh and Noor 2000) |
| Ground beef patties | Potato peels, Fenugreek seeds and Ginger rhizomes | 0.05% and 0.1% | (Mansour and Khalil 2000) |
| Cooked pork patties fresh and frozen* | Sage, Basil, Thyme and Ginger | 10% | (Abd El-Alim et al. 1999) |

* Also measured by peroxide value

2.8 Thermal inactivation

Thermal processing has been used as a primary method for food sterilization by the application of intense heat, to destroy both spoilage and pathogenic organisms and eliminating pathogens. However, heat resistant bacteria, toxins or spores may resist heat treatment (Sprenger and Richard 1998, Juneja et al. 2001). Thermal destruction of pathogens is a time–temperature-dependent process, which is depend on their *D-value* (decimal reduction time) which is the time required at a specific temperature to destroy 90% of the organisms present (Juneja and Marmer 1998, Bellara et al. 1999). The rate of kill of the microbial population generally follows first order kinetics: that is defined as the reduction in the log number of bacterial survivors, which occurs in a linear manner over time at a given temperature. It is used to integrate the lethal effect of temperature within a given thermal process (Tomlins and Ordal 1976, Murphy et al. 2000). This approach has served the food industry for over 50 years and, to date forms the basis of most commercially used thermal processes. In spite of that, during the same period of time, deviations from first order inactivation kinetics have been frequently notes (Jackson et al. 1996). According to Sprenger and Richard (1998) and Carl and Tareq (2009), many factors can influence the thermal inactivation process including type of strain, initial microbial number, temperature and pH of the food, and the presence of protective substances such as protein and fat. The traditional log-linear thermal-death-time model generally gives a good result for thermal inactivation of microorganism only in situations when the death rate is high.

2.9 Vacuum packaging (VP) and modified atmosphere packaging (MAP) enhance meat safety and quality

Considering the consumer demand for safe and high quality meat and meat products, vacuum packaging (VP) and modified atmosphere pack (MAP) are used as a solution for most ready to eat (RTE) foods. Additionally the food industry continues to apply new packaging technologies to preserve foods, by extending their shelf life (Day 1999). The purpose of meat packaging is to preserve meat and meat product with high quality and safety from the time of manufacturing to the time of consumption (Yam et al. 2005, Bhat and Hina 2011).

The effectiveness of VP and MAP derive firstly from the removal of oxygen or altering the gas atmosphere composition surrounding the food which consequently prevents the growth of oxidative microorganisms and extend the product shelf- life (Food Standards Agency 2008). However, the extension of product shelf-life throughout the using of VP or MAP always requires the additional control of other product characteristics features including pH, a_w and storage temperature (Food Standards Agency 2008). In addition, the gases and their concentrations, the packaging methods and the equipment used must be carefully controlled to guarantee product safety (Peck et al. 2006).

An important point which must be considered for storage under anaerobic conditions is the possibility of allowing anaerobic pathogenic bacteria such as *Clostridium botulinum* to grow (Gould 2000).

In MAP, different levels of oxygen (O_2) and carbon dioxide (CO_2) are used in balance with inert nitrogen (N_2). The O_2 maintains the bright red colour of meat that consumers demand (McMillin et al. 1999), and the CO_2 acts as an

antimicrobial agent by slowing down the growth of spoilage bacteria and extends the shelf life of the product (Mohn 2000).

According to Ray (2005), CO₂ increases the lag and exponential phases of microorganisms by rapid alteration of the cell permeability, reduction the pH inside the cells due to the solubilisation of CO₂ to carbonic acid (H₂CO₃) and finally, interference of CO₂ with enzymatic and biochemical pathways, leading to slowdown the microbial growth rate.

The efficacy of CO₂ to inhibit the microbial growth occurs at a level of 10% and increased when higher levels of CO₂ were used. Too high a concentration of CO₂ can inhibit the growth of facultative spoilage bacteria and encourage the growth of *Clostridium botulinum* (Gill and Molin 1990). Church (1994) reported that Gram-negative bacteria are generally more sensitive to CO₂ than Gram-positive bacteria (Smith et al. 1990).

The safety and the shelf-life of vacuum packed and MAP chilled foods with respect to non-proteolytic *C. botulinum* have been reported by the Food Standards Agency (2008) in the United Kingdom, summarising the recommendations of the Advisory Committee on the Microbiological Safety of Food as follows:

1. For products with a shelf-life of more than 10 days, besides storage at chill temperatures, the following control factors should be used either singly or in combination to inhibit the growth of non-proteolytic *C. botulinum*, including heat treatment of 90°C for 10 minutes or equivalent, pH of 5 or less, minimum salt level of 3.5%, and a_w of 0.97 or less.
2. The shelf-life must be less than 10 days and the product must be stored at 8°C or below, if the demonstrated controlling factors are absent.

Viana et al (2005), indicated that MAP not only increases the shelf life of meat and meat products, but also improves other quality aspects such as colour stability due to the effect of packaging atmosphere on myoglobin redox chemistry, which is the primary determinant of cooked meat colour (Hunt et al. 1999).

According to Ordonez et al (1991) and Jackson et al (1992) high oxygen levels in MAP enhanced the growth of different types of spoilage bacteria in meat and meat products . Thus, bacterial strains can be inhibited more or less by using CO₂. For example pure CO₂ (100%) extends the shelf life and prevents off-odour in pork meat stored at 4°C for 40 days, while in oxygen packaging the shelf life is reduced to only 10 days (Blickstad et al. 1981). However, the major disadvantages of using high CO₂ concentrations in MAP meat and meat products are associated with a certain degree of darkening in colour, resulting from metmyoglobin formation (Gill and Jones 1996). Successful packaging technique could be obtained by combination of chill temperature with MAP that generate more effective safe storage, with long shelf life (Leistner and Gorris 1995).

2.10 Hazard analysis as a tool for controlling meat safety

Hazard analysis Critical Control Point (HACCP) is a powerful procedure for ensuring quality and safety meat products (Phillips 1998). It is an efficient approach for applying knowledge of microbiological concept by identification and assessment of hazard and risk within the food operation and evaluates all procedures in the production, distribution, storage conditions that contribute to an increased risk of food-borne illness (Notermans et al. 1995, Doménech et al. 2007) (Figure 2. 3).

The hazards could be biological, chemical, and physical (World Health Organization 2002, Food and Drug Administration 2005). Successful hazard management is dependent upon the transformation of research derived knowledge into practical production, processing and food preparation practices (Miller et al. 1998). Under HACCP programs, microbiological tests more commonly track amounts of generic bacteria that are naturally found in measurable quantities on raw meat, that can be an indicator of how well a factory is succeeding in reducing the much rarer and harder to find pathogenic strains (American Meat Institute 2001, World Health Organization 2002).

According to the Chilled Food Association (2010), HACCP and monitoring checks in RTE food in respect to *L. monocytogenes*, including:

- Process validation, verification and monitoring (e.g. temperature, time, pH and aw)
- Ingredients traceability and microbiological quality testing including for hygiene indicator organisms and/or *L. monocytogenes*
- Sampling for *Listeria* species and appropriate hygiene indicator organisms from processing areas and equipment (to demonstrate the efficacy of factory hygiene and cleaning regimes)

- Final product testing for *L. monocytogenes* for example on the day of production and/or at the end of shelf life to verify effective functioning of the HACCP system and durability verification

Hazard analysis critical control point (HACCP) system is an important aspect to identify the risk assessment in RTE meat product. Quantitative risk assessment is a tool, which can be used to determine human exposure to pathogens and the probability of such organisms causing illness. Storage tests, microbiological challenge testing and mathematical models are used to predict growth or death of these organisms during processing and post-distribution handling and thereby provide information on the risk of human exposure to pathogenic micro-organisms at the time the food is consumed (Notermans et al. 1995).

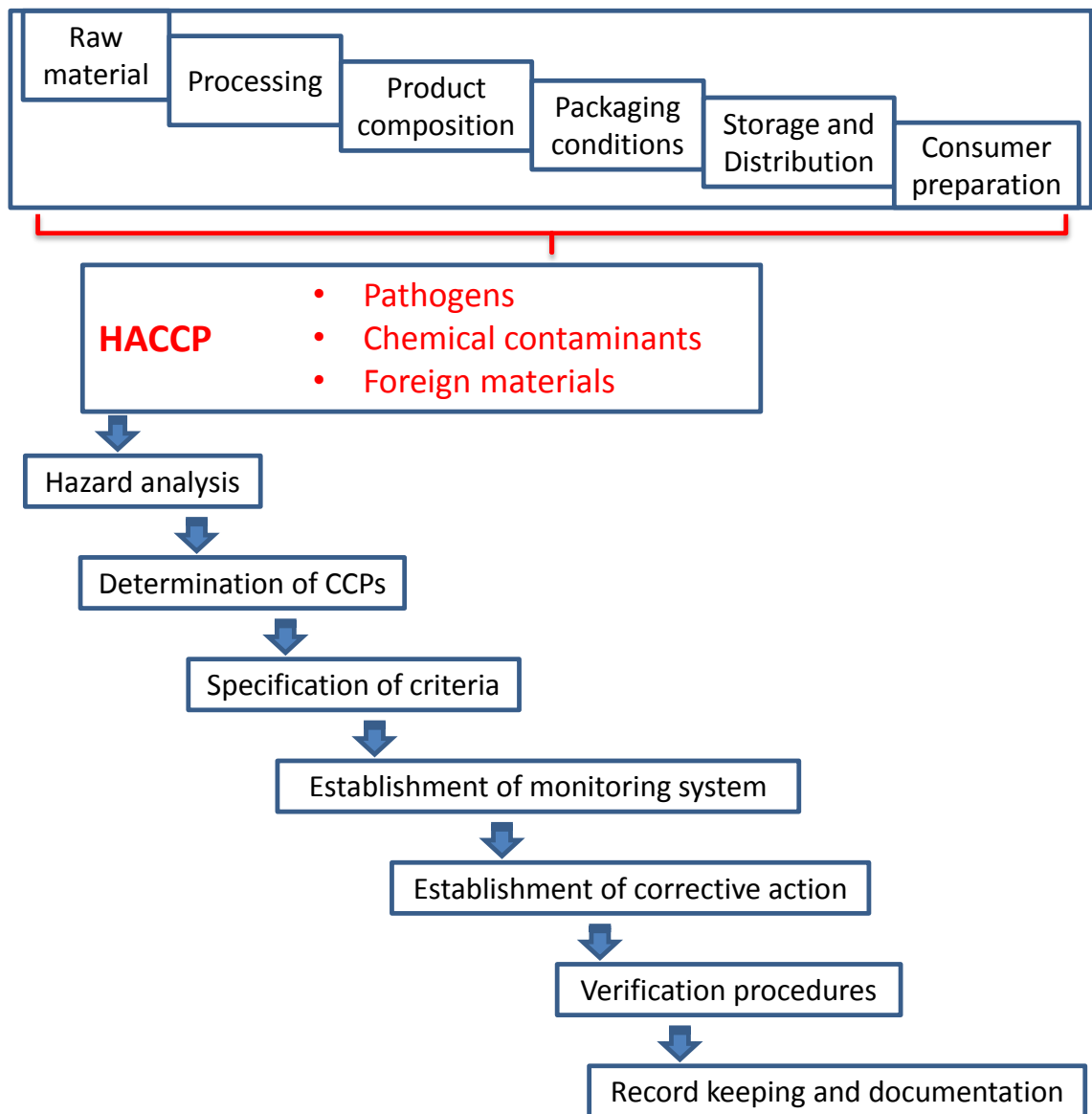


Figure 2.3: The hazard analysis critical control point (HACCP) concept and its seven basic principles.

2.11 Predictive bacterial growth

Predictive mathematical model is a promising field in food microbiology to assess the risks associated with pathogenic and spoilage bacteria in food during processing, distribution, storage condition and handling, and also help to design control measures to produce safe and good quality meat products (Koutsoumanis et al. 2005, Nychas et al. 2008). These models are built with data from laboratory testing and computer software to mathematically describe the growth kinetic parameters of microorganisms over a range of specific intrinsic and extrinsic conditions (Francois et al. 2005, Food Safety Authority of Ireland 2011).

Predictive mathematical models are a useful method for estimating the shelf life of the products, particularly in the early stages of product development and when the products are subject to minor processing or formulation changes. Although, predictive models can offer a cost effective solution by reducing the microbiological testing to determine product shelf-life (Buchanan 1993), some models may not be precise, due to inconsistent microbial responses and/or variations in the growth media (Food Safety Authority of Ireland 2011).

Many factors could influence the behaviour of pathogenic and spoilage bacteria in food such as the initial bacterial count of microorganisms and the environmental conditions. Thus, models should be built and developed to describe bacterial growth, inactivation and survival over a range of different conditions in food (Banja 2010, Havelaar et al. 2010).

2.12 Conclusion

Food-borne disease resulting from consumption of meat contaminated with pathogenic bacteria is still one of the leading causes of morbidity. Advisory

Committee on the Microbiological Safety of Food reported that doner kebabs are specifically mentioned as suspected vehicles of infection in England and Wales (Advisory Committee on the Microbiological Safety of Food 2004).

Cross contamination occurring within the premises, as well as no proper time–temperature control for heating, cooling and storage are considered as a main cause of doner kebab contamination (Gordon et al. 1995, Advisory Committee on the Microbiological Safety of Food 2004).

Doner kebab is commonly assembled in big pieces for cooking, where heating up and cooling down is relatively slow, and could allow spore-forming bacteria to grow particularly *Clostridium perfringens*. *C. perfringens* is the commonest form of food poisoning associated with cooked meat products, thus doner kebab contaminated with this type of pathogenic bacteria could represent a potential risk of hazard for public health (Todd et al. 1986, Gordon et al. 1995).

Moreover, doner kebab had a high level of fat in their composition that could be prone to rapid oxidation. Therefore, in order to improve the quality and microbiological safety of doner kebab, different aspect of this type of product must be studied. These include identification of hazard analysis to determine critical control points, application of novel preservative methods to extend the shelf life, and the use of mathematical models to predict the growth kinetic parameters of pathogenic and spoilage bacteria.

One of the preservation techniques that are attractive to consumers is the use of natural antioxidant and antibacterial compounds from spices and herbs. Using spices and herbs as food preservatives is imperative in the current world environment in which the quality and safety of meat products are of major importance; however, their effect on high fat meat product and on the sensory properties must be studied.

Chapter 3

3 General materials and methods

3.1 Natural extracts

Rosemary, cinnamon, cloves, black pepper and spearmint extracts: two different commercial types of rosemary, cinnamon, cloves, black pepper extracts and one of spearmint were provided by Kalsec (Kalsec Mildenhall, UK): oleoresin and aquaresin for all extracts except spearmint which was only aquaresin see Table 3.1.

Table 3.1: Spice extracts types and code number.

| Spice extracts | Code | Volatile oil |
|----------------------------------|-----------|--------------|
| Aquaresin black pepper, NS (BPA) | 03-21-39 | 7.5-10% |
| Oleoresin black pepper (BPO) | 03-01 | 15-20% |
| Aquaresin cinnamon, NS (CA) | 29-01-39 | 12-20P% |
| Oleoresin cinnamon (CO) | 29-01 | 25-40% |
| Aquaresin clove, NS (CLA) | 05-03-39 | 20-30% |
| Oleoresin clove, NS (CLO) | 05-03 | 40-60% |
| Aquaresin oil of spearmint (MA) | 38-817535 | 40-68% |
| Aquaresin rosemary, NS (RA) | 19-06-39 | 1-2% |
| Oleoresin rosemary, NS (RO) | 19-06 | 2-4% |

Sumac powder was obtained from Green Cuisine Food Products (Green Cuisine Watton, UK) and two types of extracts were prepared (aqueous and hydro alcoholic extracts).

3.1.1 Preparation of sumac extract

3.1.1.1 Aqueous extract

Sumac water extract (SA) was prepared by adding 5 g of sumac powder to 95 ml distilled water, left for 1 hour at room temperature with occasional stirring followed by boiling for 2 min. The extract was obtained by filtration through Whatman # 4 filter paper according to Nasar-Abbas and Halkman (2004).

3.1.1.2 Hydro alcoholic extract

Hydro alcoholic extracts of the sumac (SO) was prepared according to Fazeli et al. (2007) by a percolation method using 80% (v/v) aqueous ethanol. Extracts were concentrated in a vacuum rotary evaporator (Buchi, Switzerland) and were left to dry in desiccators, the extracts were stored in a dark cold place for 2-3 weeks at refrigerated temperature (4±1°C).

3.2 Bacterial cultures

Listeria monocytogenes 5105, *Listeria innocua* 4202, *Lactobacillus sake* 550, *Staphylococcus aureus*, *Salmonella* Typhimurium DT104, *Escherichia coli* K12, *Pseudomonas aeruginosa* B 8626, *Bacillus cereus* NCIMB 11925, *Bacillus subtilis* and *Clostridium perfringens* NCTC 8239, cultures were obtained from the School of Biomedical and Biological Sciences / University of Plymouth culture collection.

Listeria monocytogenes NCTC7973 (type strain) was obtained from the national collection of type cultures (Health Protection Agency Culture Collection, Centre for infections, London, UK).

3.3 Culture media

All Media and Supplements (Table 3.2) were obtained from (Oxoid Ltd., Basingstoke, Hampshire, UK) and were prepared according to the manufacturers' instructions. Media were sterilized by autoclaving (121 °C for 15 min).

Table 3.2: Culture media and supplements.

| Culture media | Supplement |
|---|--|
| Bacillus cereus selective agar Base (PEMBA) CM0617 | Polymyxin B Supplement SR0099 |
| Baird–Parker agar (BP) CM0275 | Egg Yolk Tellurite Emulsion SR0054 |
| Brain heart infusion agar (BHI) CM1135 | - |
| Brilliant green agar (BGA) CM0263 | - |
| Buffered Peptone Water CM0509 | - |
| De Man, Rogosa, Sharpe agar (MRS) CM0361 | - |
| Fraser broth CM0895 | Fraser Selective Supplement SR0156 |
| Half Fraser broth CM0895 | Half Fraser Selective Supplement SR0166E |
| Listeria selective agar (Oxford agar) CM0856 | Modified Listeria Selective Supplement (Oxford) SR0206 |
| Maximum recovery diluent (MRD) CM0733 | |
| Nutrient broth (NB) CM0001 | - |
| Perfringens agar (OPSP) CM0543 | Perfringens (OPSP) Selective Supplement A SR0076 Perfringens (OPSP) Selective Supplement B SR0077 |
| Plate count agar (PCA) CM0325 | - |
| Rappaport-vassiliadis soya Peptone broth (RVS) CM0866 | - |
| Rose Bengal Chloramphenicol Agar (RBCA) CM0549 | Chloramphenicol Supplement SR0078 |
| Tryptone Bile agar (TBX) CM0595 | - |
| Violet Red Bile Glucose agar (VRBGA) CM0485 | - |
| Xylose lysine desoxycholate agar (XLD) CM0469 | - |

3.4 Preparation of inoculum

A loop full of inoculum was taken from a pure culture of the respective bacteria grown on plate and inoculated into 10 ml nutrient broth (Oxoid Ltd., Basingstoke, Hampshire, UK). The broth suspension was incubated for 18 hour aerobically at 37°C (LEEC incubator, LEEC Limited, Colwick Industrial Estate, Nottingham, UK), except *Pseudomonas aeruginosa* which was incubated at 30°C (Swallow Incubators, LTE Scientific Ltd, Green field, Oldham, UK). Broth suspension containing *Clostridium perfringens* and *Lactobacillus sake* were incubated anaerobically at 37°C using an anaerobic incubator (Modular Atmosphere Controlled System, Don Whitley Scientific Limited, West Yorkshire, UK).

3.5 Preparation of normal saline

Saline Tablets BR0053 (Oxoid Limited, Basingstoke, Hampshire, UK) were used by dissolving one tablet in 500 ml of distilled water to obtain 0.85% normal saline.

3.6 Preparation of maximum recovery diluent (MRD)

Maximum recovery diluent (MRD CM0733, Oxoid Ltd., Basingstoke, Hampshire, UK) was prepared according to the manufacturers' instructions by dissolving 9.5g of base in 1 litre of distilled water, dispensed into the final containers and sterilised.

3.7 Preparation of nutrient broth (NB)

Nutrient broth (NB CM001, Oxoid Ltd., Basingstoke, Hampshire, UK) was prepared according to the manufacturers' instructions by dissolving 13g of base in one litre of distilled water then distributing in 10ml aliquots into universal

tubes, using an Eppendorf dispenser (Eppendorf AG, 22331, Hamdarg, Germany) and autoclaved at 121°C for 15 min.

3.8 Preparation of brain heart infusion agar (BHI)

Brain heart infusion agar (BHI CM 1135, Oxoid Ltd., Basingstoke, Hampshire, UK) was prepared by dissolving 37g of base in one litre of distilled water microwaving for 10 min, then distributed in 20 ml aliquots in universal tubes using an Eppendorf dispenser (Eppendorf AG, 22331, Hamdarg, Germany). All tubes were sterilized by autoclaving for 15 min at 121°C and used to prepare agar plates for the agar well diffusion method.

3.9 Ethical approval

The Human Ethics committee of the Faculty of Science /University of Plymouth approved the sensory evaluation protocol. Each participant was provided with a consent form. The consent form stated that each participant has the ability to withdraw from the panel at any time, without penalty or having to give a reason during the experiment.

3.10 Doner kebab sample

3.10.1 Commercial doner kebab

Raw doner kebab composed of lamb meat, fat, rusks, onions, spices, salt, herbs, durum, monosodium glutamate and stabilizer-E450 was purchased from K B S Factory (Cardiff, UK) and kept in a freezer at -18°C until used.

3.10.2 Manufactured doner kebab

Lamb meat, lamb fat and rusk were purchased from local butchers (City centre, Plymouth). Doner kebab was manufactured in the food and nutrition lab according to a simplified commercial formula: 70% lamb meat, 28% lamb fat, 1% rusk, 0.5% salt and frozen until use. To get the base mixture, lamb

trimmings were ground through a 5-mm plate in a mincing machine (ModEM20, Crypto Peerless mincer, Birmingham, England). Afterwards the other ingredients were added into the bowl mixer and mixed with the spiral dough hook (Spiral dough hook, Silverson, Birmingham, UK) at medium speed (80 rpm) for 5 min.

3.11 Prepared and Cooked doner kebab slices

Raw doner kebab was thawed over night at $4\pm1^{\circ}\text{C}$ and shaped to slices using a plastic tray and knife, the thickness of each slice was 5mm, while the length and the width were 10-5 mm respectively. The slices then were cooked using a gas oven (Zanussi Combi Wave -Zanussi Grandi Impiantispa- Italia) at 200°C for 8 minutes to reach an internal temperature of 72°C in the centre of the kebab slices. The temperature of the oven and the geometric centre of some doner kebab slices was monitored continuously using thermocouple probes (K type), which were connected to a temperature logger (Comark Electronics, Ltd., Littlehampton, UK).

3.12 Storage conditions

A medium gas barrier, vacuum pouch produced from coextruded film made of polyamide and polyethylene sealing layer was used (thickness 70 microns, water vapour permeability $3\text{ g/m}^2\text{ 24h}$, oxygen permeability $50\text{ cc/m}^2\text{ 24h}$, CO_2 permeability $150\text{ cc/m}^2\text{ 24h}$ and N_2 permeability $10\text{ cc/m}^2\text{ 24h}$). The bags were either vacuum then sealed (VP) or a gas mixture of (70% N_2 and 30% CO_2) was pumped in to the bag (MAP) unless stated otherwise and sealed using (Multivac MU 61, Germany) for VP and MAP respectively. The slices were stored in a cooler at $4\pm1^{\circ}\text{C}$ (Williams Refrigeration and Air-conditioning, installation by general refrigeration, Limited) during the storage time

3.13 Doner kebab analysis

3.13.1 Microbiological analysis

Doner kebab samples were taken aseptically for microbiological analysis, a composite sample (10 g) was formed with portions of at least 3 doner kebab slices and homogenised with 90 ml sterile 1.5% peptone water, in a Stomacher 400 (Seward Medical, London, UK) for 1 min.

Aliquots were serial diluted in maximum recovery diluent (MRD) and plated out following National Standard Methods (Labbe 2001, Health Protection Agency 2004d, 2009c). Lactic acid bacteria counts (LAB) were determined on De Man, Rogosa, Sharpe Agar (MRS, pH 5.6), with the plates incubated under 5% CO₂ incubator at 37°C for 48 hours. Total viable counts were determined on plate count agar (PCA) with plates incubated aerobically at 30°C for 24-48 hours. Enterobacteriaceae were enumerated on Violet Red Bile Glucose Agar (VRBGA) then the plates were incubated at 37°C for 24 hours (Health Protection Agency 2005). Mould and yeast counts were determined on Rose Bengal Chloramphenicol Agar (RBCA) at 25°C for 5 days. *Staphylococcus aureus* was enumerated on Baird–Parker agar (BP) and incubated at 37°C for 48 hours (Health Protection Agency 2004c). *E. coli* was enumerated on Tryptone Bile agar (TBX), plates and incubated at 37°C for 24 hours.

A composite of 25 g cooked doner kebab sample was formed with portions of at least 3 doner kebab slices to determine the presence/absence of *L. monocytogenes* (Health Protection Agency 2009a). For detection of *L. monocytogenes*, each sample was homogenised with 225 ml of half Fraser broth (primary enrichment medium) at 30°C for 24h; then 0.1 ml of primary enrichment broth was transfer to a secondary enrichment media (Fraser broth) which was incubated for 48h at 37°C, then the culture was directly sub-cultured

onto *Listeria* selective agar (Oxford agar, Code: CM0856) for 48h at 37°C for both enrichment stages. Oxford agar was examined for the presence of typical colonies and identification of the species by means of morphology on blood agar (beta haemolysis), motility test and biochemical tests that include; rhamnose and xylose sugar fermentation.

For enumeration of *L. monocytogenes*, the homogenised sample was directly inoculated on the surface of Oxford agar with a 0.5 ml of a 10^{-1} and other appropriate decimal dilutions, and then plates were incubated at 37°C for up to 48 hours. Calculation of the number of CFU per gram of sample for *L. monocytogenes* was made from the number of typical colonies obtained on Oxford agar, and subsequently confirmed by morphological and biochemical tests (Leguerinel and Mafart 2001).

For detection of *Salmonella* spp., pre-enrichment of a composite of 25 g cooked doner sample from portions of at least 3-doner kebab slices were homogenised with 225 ml of sterile 1.5% buffered peptone water, in a Stomacher 400 (Colworth, London, UK) for 1 min. and incubated at 37°C. After 24hours, 0.1 ml was taken from the pre-enrichment broth and cultured into 10 ml selective enrichment broth Rappaport-vassiliadis soya Peptone broth (RVS broth), which was incubated for 24hours at 42°C. A loop full of enrichment broth was sub cultured by streaking onto plates of xylose lysine desoxycholate agar (XLD) and brilliant green agar (BGA); then plates were incubated at 37°C for 24 hours (Health Protection Agency 2008). The colony counts were log transformed and expressed as \log_{10} CFUg⁻¹ of doner kebab before being statistically analysed.

3.13.2 Biochemical analyses

3.13.2.1 Rancimat method (stability index)

The antioxidant stability index was measured by using the American oil chemist society (AOCS) air oxidation method. All experiments were performed with a 679 Rancimat (Metrohm, Herisau, Switzerland).

Procedure: the dry and wet section of the Rancimat instrument were turned on, with the air set at 20 L^h and the heat set at 120°C for one hour before starting the experiment. A sample of 3.25 ± 0.01 g was inserted into the reaction tube and a glass ring inserted into each tube prior to the reaction tube attachments being secured to the tube with the aid of ground glass joint clamps. When the set temperature was reached, the tubes were placed into the cavities on the heating block and then connected to the measuring vessels by joint clamps. The samples were then heated without air supply for 10 minutes to allow the temperature to stabilise. The reaction tubes were then connected to the air supply and the air rate adjusted to the desired value. The conductivity was measured and plotted constantly. Three replications of this experiment were made.

3.13.2.2 TBA values

The secondary lipid oxidation products from cooked doner kebab were assessed by the 2-thiobarbituric acid (TBA) method of Tarladgis (1960) with minor modifications (distillation method) using a Vapodest 40 - Programmable distillation unit for Kjeldahl digestion (Gerhardt, Dublin). A 10 g sample was homogenised with 80 ml distilled water for 1 min in a Stomacher 400 (Seward Medical, London, UK) and then transferred to a distillation flask. The bag used for blending washed with additional 17.5 ml distilled water, which was added to the same distillation flask with 2.5 ml 4N HCl and few drops of anti-foam agent

silicone o/w (Fisher Scientific, Loughborough, UK). The mixture was distilled and 50 ml distillate was collected. Five ml of 0.02 M 2-thiobarbituric acid in 90% acetic acid (TBA reagent) was added to a vial containing 5 ml of the distillate and mixed well. The vials were covered and heated in a boiling water bath for 30 minutes for colour development and cooled at room temperature for 10 min. Then 300 μ L of the mixture was dispensed into clean 96-well microplate to measure the absorbance at 538 nm using a plate reader spectrophotometer (Spectra Max 340PC, Molecular Devices Corporation, Sunnyvale CA, USA). A blank was prepared with 5 ml distilled water and 5 ml TBA-reagent. TBA value was evaluated weekly during storage time. A minimum of two samples were taken with three readings in triplicate wells. Thiobarbituric acid-reactive substances (TBARS) were calculated from a standard curve (1×10^{-8} to 7×10^{-8} mol) of malondialdehyde (MA), freshly prepared by acidification of 1, 1, 3, 3-tetraethoxy propane (TEP) (Sigma, Poole, UK). The TBA numbers expressed as mg MA/kg sample.

3.13.2.3 pH value

The pH value of doner kebab samples was determined weekly by using a microprocessor pH meter (pH 213, HANNA Instrument, Indonesia). Ten g of ready to eat doner kebab slices were each homogenised thoroughly for 30 seconds with 100 ml of distilled water using a stomacher 400 (Seward Medical, London, UK). The homogenate was then used for pH determination.

3.13.2.4 Water activity

Water activity for cooked doner kebab samples was determined weekly for 11 weeks in duplicate at room temperature (22°C) using a Novasina Thermoconstanter, Novasina Aw Sprint TH-500 (Novasina, Switzerland). Samples were transferred to a measuring cell until about half full, and then the

measuring cell was left in the instrument until constant readings indicated that equilibrium was reached.

3.13.3 Sensory analysis

Sensory evaluation followed British Standard Guidelines ISO 6658-1995 and 6564-1985 (British Standards 5929 1986a, b) was used.

3.13.3.1 Scoring test (Hedonic scale)

This evaluation was performed on the refrigerated doner kebab during storage time to determine the shelf life (quality) of the products by quantify the change in colour, aroma, texture ,overall appearance and overall acceptance (Morten Meilgaard et al. 1999) (Appendix B1 & B2).

A six semi-trained member panel (Staff and postgraduates of Plymouth University) evaluated a randomly coded sample. Panellists asked to heat the sample in a microwave oven (Brother Hi- Speed Combination Cooker / Japan) before evaluation of the sample. The sensory tests measured intensity on a 7-point balanced hedonic scale, where seven = very acceptable and one = very unacceptable for the following attributes colour, odour, texture, overall appearance and overall acceptance

3.14 Statistical analysis

Unless stated otherwise, all the numerical data were analysed using MINITAB version 15 (Minitab Ltd, Coventry, UK), one-way analysis of variance (ANOVA), and general linear model (GLM) were used to compare different treatment groups followed by one of the appropriate multiple comparison test (contrasts Tukey and LSD 5%). Data are shown as means +/- SE and $P < 0.05$ is considered significant.

Chapter 4

4 Comparison of the Antibacterial and Antioxidant Activity of Oil and Water Extracts of Culinary Spices

4.1 Introduction

Spices have been added to food as flavouring agents since ancient times to impart flavour, and piquancy (Ockerman and Basu 2004). Spices and herbs contain many phytochemical compounds such as phenolic diterpenes, flavonoids, tannins, and phenolic acids which are considered potential sources of natural antibacterial, antioxidant, anti-inflammatory substances as well as having anticancer activity (Kenji and Mitsuo 1998, Juneja and Friedman 2007, Kaefer and Milner 2008, Rahman and Kang 2009).

Food-borne diseases as well as food deterioration are major problem for researchers and food processing manufactures. Therefore, preventing food cross contamination with pathogenic and spoilage bacteria were considered the main goal to obtain safe and good quality food (Nissen et al. 2004).

In addition, lipid oxidation is one of the basic processes causing rancidity in food products, which is characterized by formation of a number of undesirable compounds leading to changes in flavour, colour, and smell as well as texture (Tikk et al. 2008, Cava et al. 2009). This primary process occurs due to auto oxidation of lipid substances to produce peroxides, which are further oxidized and decompose resulting in quality deterioration in foods, and especially in long-life or precooked meat products. In an effort to prevent lipid oxidation, and due to the link between lipid oxidation and health, therefore, researchers and food scientists have been dealing with different antioxidants for a long time.

Global interest in bio-preservation of food systems, particularly of plant origin such as spices and herbs has recently been increased due to consumer demand for safe and healthy food free from synthetic additives (Wellwood and Cole 2004, Hernández-Hernández et al. 2009). Consequently, the development and application of natural products with antibacterial and antioxidant properties in meat products may be necessary to prolong their storage shelf life and potential for preventing food-borne diseases (Ceylan et al. 2004, Shan et al. 2007b).

Rosemary (*Rosmarinus officinalis* L.) has been reported to contain certain compounds including, rosmanol, rosmariquinone, rosmaridiphenol and carnosol, which may be up to four times as effective as butylated hydroxy anisole (BHA) (Kenji Hirasa 1998) and equal to butylated hydroxy toluene (BHT) as antioxidants (Cuvelier et al. 1996, Kenji and Mitsuo 1998). Additionally, several authors reported that some compounds present in rosemary extracts could have antibacterial activity (Giorgio et al. 2002, Fernández-López et al. 2005). These antimicrobial activities are mostly attributable to the presence of phenolic di-terpenoids, which are the main compounds of the polar fraction of the rosemary extracts (Shan et al. 2007b).

Cinnamon (*Cinnamomum zeylaicum* L.) belongs to the family Lauracea. Cinnamon bark and oil have been used as food additives, condiments and flavouring agents due to their carminative, antioxidant and preservative actions. It has been found that ethyl cinnamate, eugenol, cinnamaldehyde, beta-caryophyllene, linalool, and methyl chavicol are the main essential oils responsible for the antimicrobial and antioxidant properties (Shan et al. 2007b, Paparella et al. 2008).

Sumac (*Rhus coriaria* L., family Anacardiaceae) is widely used in the Middle East region. The fruits are red coloured and contain one seed which is dried and ground to use as a sprinkled condiment over kebab, grilled meat and salad. Sumac has an acidic sour taste due to citric and malic acid found in their composition. It has been reported that sumac contains high concentrations of anthocyanins and hydrolysable tannins which are responsible for sumac oxidative property (Kosar et al. 2007). In addition, several authors reported that the phenolic compounds especially gallic acid possesses antimicrobial activity against several types of bacteria (Nasar-Abbas and Halkman 2004, Gulmez et al. 2006, Fazeli et al. 2007).

Clove (*Eugenia caryophyllata* L. family Myrtaceae) is widely used in industries as a fragrance and flavouring agent. Phenylpropanoids are the important constituents of the essential oil which includes carvacrol, thymol, eugenol and cinnamaldehyde (Kamel et al. 2007). As reported by Jirovetz et al. (2006), clove essential oil contains eugenol (76.8%), followed by beta-caryophyllene (17.4%), alpha-humulene (2.1%), and eugenyl acetate (1.2%) as the main components. Previous study have been reported the antimicrobial efficacy of clove against different types of pathogenic bacteria (Friedman et al. 2002, Cressy et al. 2003). Furthermore, clove extracts showed a considerable inhibitory effect against hydroxyl radicals and acted as an iron cheater, thereby prevent lipid peroxidation (Jirovetz et al. 2006).

Black pepper (*Piper nigrum* L., family Piperaceae) grows in the tropical regions especially in south India. Dried ground pepper is one of the most common spices. In Europe, it is used for flavouring and seasoning of food. It has been shown to have antimicrobial and antioxidant activity (Dorman and Deans 2000). The antioxidant properties of black pepper are due to the acidic fraction of its

extracts and the phenolic compounds (Kenji and Mitsuo 1998). Gurdip et al.(2004), reported that black pepper oil exhibited a better antioxidant activity than BHT and BHA in linseed oil.

Spearmint (*Mentha spicata* L) belongs to the family Lamiaceae that are the most popular herbal remedies all over the world and it is characterized by its distinctive flavour. Spearmint leaves are used fresh or dried for flavouring meat, fish, sauces, soups, vinegar, tea, and tobacco. Spearmint oil and dried plants have antioxidant properties (Politeo et al. 2006, Padmini et al. 2008).

The aims of this study were to compare eleven spice extracts; rosemary oleoresin, rosemary aquaresin, cinnamon oleoresin, cinnamon aquaresin, clove oleoresin, clove aquaresin, black pepper oleoresin, black pepper aquaresin, spearmint aquaresin, sumac aquaresin and sumac hydro alcoholic extract for their:

- Antimicrobial activities by using agar well diffusion method to determine the inhibitory effect and the activity unit of these extracts against pathogenic and spoilage bacteria
- Antioxidant properties were determined as stability index in lamb fat model

4.2 Material and Methods

4.2.1 *Natural extracts*

Spice extracts were provided by Kalsec and Green Cuisine Food Products as detailed in Section 3.1, and sumac extracts (hydro alcoholic and aqueous extracts) were prepared as detailed in Sections 3.1.1.

4.2.2 *Bacterial cultures*

The ten food-borne and spoilage bacteria used as test organisms were: *Listeria monocytogenes* 5105, *Listeria innocua* 4202, *Lactobacillus sake* 550, *Staphylococcus aureus*, *Salmonella* Typhimurium DT104, *Escherichia coli* K12, *Pseudomonas aeruginosa* B 8626, *Bacillus cereus*, *Bacillus subtilis* and *Clostridium perfringens* NCTC 8239. These bacteria were obtained from the School of Biomedical and Biological Sciences / University of Plymouth liquid nitrogen culture collection.

4.2.3 *Preparation of standard (McFarland's) opacity tube*

McFarland standard were made by mixing specific amounts of 1% barium chloride with 1% sulphuric acid (Harrigan 1998).

4.2.4 *Preparation of normal saline*

Normal saline was prepared as in Section 3.5 and used to dilute the bacterial culture to reach a concentration of 10^7 - 10^8 CFUml⁻¹.

4.2.5 *Preparation of nutrient broth (NB)*

Nutrient broth was prepared as in Section 3.7. These were used to prepare stock cultures of all pathogenic and spoilage bacteria.

4.2.6 *Preparation of brain heart infusion agar (BHI)*

Brain heart infusion agar was prepared as in Section 3.8.

4.2.7 Determination of antibacterial effect of natural extracts

The agar well diffusion method was used to determine the antimicrobial activity of spice extracts against 10 bacterial strains (Kim et al. 1995, Kuri et al. 1998, Fernández-López et al. 2005). Stock cultures of all tested bacteria were grown in nutrient broth (Oxoid Unipath Ltd., Basingtoke, Hampshire, UK) for 18 hours as detailed in Section 3.4. Final cell concentrations were standardized to 10^7 – 10^8 CFUml⁻¹ using the McFarland standards. Then, 200 µl of this inoculum was added to each universal tube containing 20 ml molten brain heart infusion (BHI) agar, mixed well and poured into a disposable petri dish (90*15mm). Six wells (6 mm diameter) were formed in each plate after the agar was solidified using a sterilized cork borer. Forty µl of each extract at these concentrations (1, 0.1, 0.01 w/v) was applied into each well and was left to diffuse for 15 – 20 min at room temperature, then incubated in the appropriate condition according to the type of bacterial strain for 24 hours. All strains were incubated aerobically at 37°C except *Pseudomonas aeruginosa* which was incubated at 30°C, while *Clostridium perfringens* and *Lactobacillus sake* were incubated an aerobically at 37°C. A control sample was prepared with 40 µl of sterile distilled water instead of spice extract. After incubation, the inhibition zone was measured in three directions using callipers and the average was calculated (Kuri, 1998). Three replications of this experiment were made.

4.2.8 Determination of activity units (AU)

Sequential two fold dilutions with sterile normal saline of the antimicrobial extracts were done in a plate and 40 µl aliquots of each dilution were used in the agar well diffusion method. The AU ml⁻¹ was the highest dilution with an inhibition diameter > 6 mm multiplied by a factor of 25. Three replications of this experiment were made.

4.2.9 Lamb fat preparation

Lamb fat was purchased from local supermarket, prepared and kept in the freezer (−18°C) until used for each measurement of Rancimat method. Lamb fat was prepared as describe below: fat was separated from the connective tissue then cut in to small pieces and heated gently by using indirect heating to inhibit any oxidation then filtered by sieving through muslin to obtain pure fat and freezing in plastic covered box at −18°C until use. This was done to reduce variability due to differences in the raw material.

4.2.10 Measuring the antioxidant activity (stability index)

The antioxidant stability index was measured by using the American oil chemist society (AOCS) air oxidation method. All experiments were performed with a 679 Rancimat (Metrohm, Herisau, Switzerland) as explained in Section 3.13.2.1. Spice extracts were added to 5 g of melted lamb fat, mixed well to obtain a final spice extracts concentrations of (0.1, 0.5, 1, and 2 % w/g). Lamb fat without extract added was used as control sample.

4.2.11 Statistical analysis

All Data were analysis statistically as detailed in Section 3.14.

4.3 Results

4.3.1 Antibacterial activity of spice extracts

The antibacterial activity of natural spice extracts against ten pathogenic and spoilage microorganisms tested by agar well diffusion method are presented in (Tables 4.1 and 4.2).

Table 4.1: Inhibitory activity^a of spice extracts^b against pathogenic and spoilage bacteria^c.

| Spice extracts | Bacterial strains | | | | | | | | | |
|----------------|-------------------|-------------|-------------|------------|------------|----------------|-------------|------------|------------|-------------|
| | Gram (+) | | | | | | | Gram (-) | | |
| | <i>Cl.p</i> | <i>L.m.</i> | <i>L.in</i> | <i>B.c</i> | <i>B.s</i> | <i>Staph.a</i> | <i>Lb.s</i> | <i>E.c</i> | <i>S.T</i> | <i>Ps.a</i> |
| CO | ++ | ++ | ++ | ++ | ++ | ++ | - | ++ | ++ | ++ |
| CA | ++ | + | ++ | + | + | + | - | + | + | ++ |
| RO | ++ | ++ | ++ | ++ | ++ | ++ | - | - | - | - |
| RA | ++ | ++ | ++ | ++ | ++ | ++ | - | + | - | - |
| CLO | ++ | ++ | ++ | ++ | + | ++ | ++ | ++ | ++ | - |
| CLA | ++ | + | + | + | + | + | + | + | + | - |
| SO | ++ | ++ | ++ | ++ | - | ++ | ++ | ++ | ++ | ++ |
| SA | ++ | - | - | - | - | - | - | - | - | + |
| MA | ++ | + | + | + | + | ++ | + | + | + | - |
| BPO | + | - | - | - | - | - | - | - | - | - |
| BPA | + | - | - | - | - | - | - | - | - | - |

^a ++: Inhibition by 0.1% of spice extract, +: Inhibition by 1% of spice extracts, - : No inhibition,

^b Spice extracts: CO: cinnamon oleoresin, CA: cinnamon aquaresin, RO: rosemary oleoresin, RA: rosemary aquaresin, CLO: clove oleoresin, CLA: clove aquaresin, SO: sumac hydro alcoholic extract, SA: sumac aqueous extract, MA: spearmint aquaresin, BPO: black pepper oleoresin, BPA: black pepper aquaresin,

^c Bacterial strains: *Cl. p*: *Clostridium perfringens*, *L. m*: *Listeria monocytogenes*, *L. in*: *Listeria innocua*, *B. c*: *Bacillus cereus*, *B. s*: *Bacillus subtilis*, *Staph. a*: *Staphylococcus aureus*, *Lb. s*: *Lactobacillus sake*, *E. c*: *Escherichia coli*, *S. t*: *Salmonella Typhimurium*, *Ps. a*: *Pseudomonas aeruginosa*.

Table 4. 2: Determination of antimicrobial activity of spice extracts at concentration 1% (w/v) against pathogens by agar well diffusion method.

| Spice Extracts | Diameter of the zones of inhibition in mm (6 mm well) | | | | | | | | | |
|----------------|---|-------------|-------------|------------|------------|----------------|-------------|------------|------------|-------------|
| | Gram (+) | | | | | Gram (-) | | | | |
| | <i>Cl.p</i> | <i>L.m.</i> | <i>L.in</i> | <i>B.c</i> | <i>B.s</i> | <i>Staph.a</i> | <i>Lb.s</i> | <i>E.c</i> | <i>S.T</i> | <i>Ps.a</i> |
| CO | 21.0 | 30.5 | 23.0 | 32.5 | 13.8 | 17.5 | - | 16 | 14.5 | 12.5 |
| CA | 20.0 | 14.0 | 21.5 | 12.0 | 10.0 | 14.8 | - | 13 | 13.8 | 10.8 |
| RO | 27.5 | 24.8 | 20.0 | 19.8 | 18.0 | 15.5 | - | - | - | - |
| RA | 14.5 | 12.0 | 12.5 | 11.0 | 8.00 | 8.75 | - | 9.25 | - | - |
| CLO | 12.0 | 10.5 | 12.0 | 13.5 | 9.00 | 11.5 | 15.5 | 11 | 12.0 | - |
| CLA | 12.0 | 11.0 | 10.5 | 10.0 | 8.50 | 8.00 | 9.50 | 9 | 9.50 | - |
| SO | 15.0 | 15.5 | 14.0 | 14.8 | - | 12.8 | 12.0 | 14.75 | 16.0 | 13.0 |
| SA | 11.0 | - | - | - | - | - | - | - | - | 9.25 |
| MA | 15.7 | 12.0 | 8.50 | 10.0 | 10.5 | 9.5 | 9.50 | 11 | 10.5 | - |
| BPO | 13.8 | - | - | - | - | - | - | - | - | - |
| BPA | 12.0 | - | - | - | - | - | - | - | - | - |

:- no inhibition; key of the table as Table 4.1

All spice extracts exhibited different efficacy depending on the type of the extracts and the concentration used. The inhibitory effect of all spice extracts were concentration dependent. The greatest antimicrobial effect was observed at a concentration of 1% followed by 0.1% (Tables 4. 1). Cinnamon oleoresin showed higher antibacterial effect than other tested extracts followed by a moderate effect by sumac alcoholic extract, cinnamon aquaresin, rosemary oleoresin, clove oleoresin, spearmint, clove aquaresin and rosemary aquaresin respectively. Black pepper and sumac water extract had the lowest antimicrobial effect (Tables 4. 1 and 4.2).

4.3.2 Activity unit (AU) ml⁻¹ and the Spectrum range

The activity unit (AU) and the spectrum range are presented in Figure 4.1. When considering the mean of activity unit (AU) of the spice extracts, cinnamon oleoresin (CO) and sumac alcoholic extract (SO) were found to be the most effective. Followed by the moderate effect which included rosemary (RO, RA), cinnamon aquaresin (CA), clove (CLO, CLA), sumac water extract (SA) and spearmint (MA), while black pepper (BPO, BPA) had the lowest effect.

Regarding the spectrum range of spice extracts against tested bacterial strains cinnamon, sumac alcoholic extract, clove aquaresin and spearmint have the wide spectrum and acted against nine bacterial strains, while clove oleoresin, rosemary aquaresin and rosemary oleoresin had a moderate effect with spectra range about eight, seven and six respectively. The lowest spectra represented by sumac water extract (2) strains and black pepper (1) strain only.

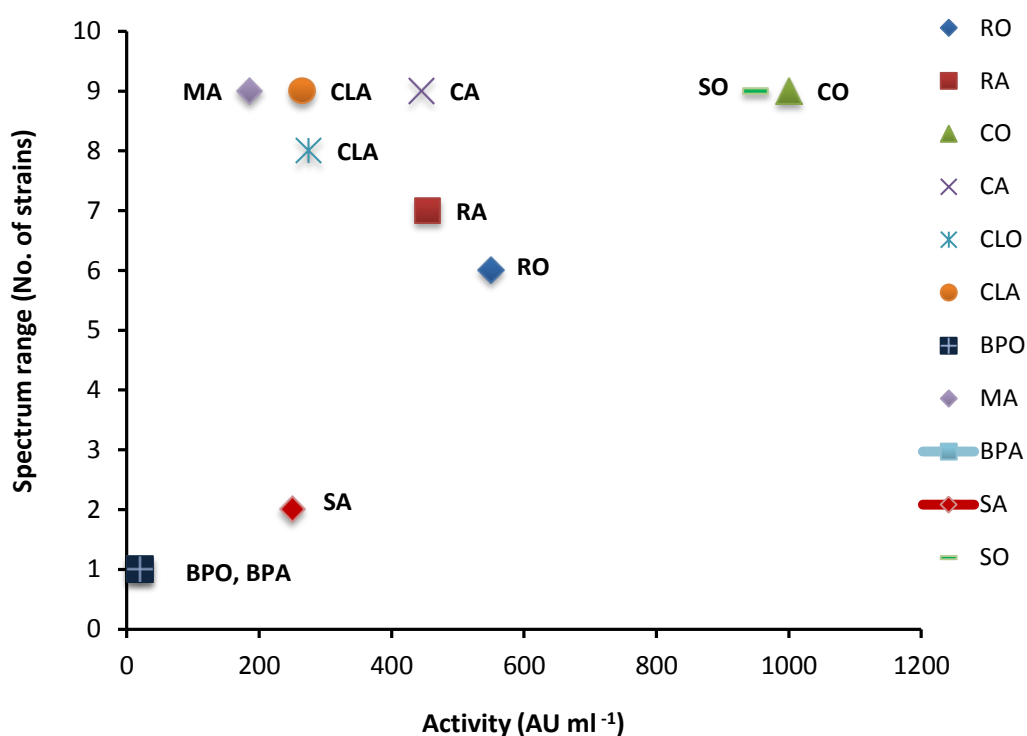


Figure 4.1: Spectrum range and activity (AU ml⁻¹) of the spice extracts against selected bacteria. Key of the figure as Table 4.1

4.3.3 Sensitivity of bacterial species tested

Bacterial species were shown variable sensitivity toward spices extracts (Table 4. 3). Generally, Gram-positive bacteria showed more sensitivity than Gram-negative bacteria to all spice extracts tested. Of the ten bacterial species tested, *Clostridium perfringens* was considered the most sensitive strain, inhibited by all spices extracts in the activity range between 200-2000 AU ml⁻¹. *Bacillus cereus*, *Listeria innocua*, *Listeria monocytogenes* and *Staphylococcus aureus* were found to be inhibited by eight extracts, followed by *Bacillus subtilis*, *Escherichia coli* and *Salmonella* Typhimurium, while *Lactobacillus sake* and *Pseudomonas aeruginosa* were the most resistant bacterial strains being inhibited by four extracts only.

Table 4. 3: Mean of activity units (AU) ml⁻¹ of spice extracts against selected bacteria.

| Spice extracts | Bacterial strain | | | | | | | | | |
|----------------|------------------|------------|------------|------------|------------|----------------|--------------|------------|------------|-------------|
| | Gram (+) | | | | | | | Gram (-) | | |
| | <i>Cl.p</i> | <i>L.m</i> | <i>L.i</i> | <i>B.c</i> | <i>B.s</i> | <i>Staph.a</i> | <i>Lb. s</i> | <i>E.c</i> | <i>S.T</i> | <i>Ps.a</i> |
| CO | 2000 | 1000 | 1500 | 1000 | 1500 | 1000 | 0 | 500 | 1000 | 500 |
| CA | 2000 | 200 | 1000 | 200 | 200 | 200 | 0 | 200 | 200 | 250 |
| SO | 1500 | 1000 | 1000 | 1000 | 0 | 1000 | 1000 | 1000 | 1000 | 1000 |
| SA | 1500 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1000 |
| RO | 1500 | 500 | 500 | 500 | 2000 | 500 | 0 | 0 | 0 | 0 |
| RA | 1500 | 500 | 500 | 1000 | 500 | 500 | 0 | 50 | 0 | 0 |
| CLO | 1000 | 250 | 250 | 250 | 0 | 250 | 250 | 250 | 250 | 0 |
| CLA | 1500 | 150 | 200 | 100 | 100 | 100 | 200 | 150 | 150 | 0 |
| MA | 500 | 200 | 100 | 150 | 50 | 500 | 100 | 150 | 100 | 0 |
| BPO | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| BPA | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Key to the table as Table 4.1.

4.3.4 Antioxidant activity of spice extracts

Antioxidant properties of spice extracts were expressed as stability Index (SI) (Figures 4.2 and 4.3). In general, all tested spice extracts had antioxidant properties with different efficacy except sumac water extract and spearmint aquaresin, which showed activity levels lower than the control sample.

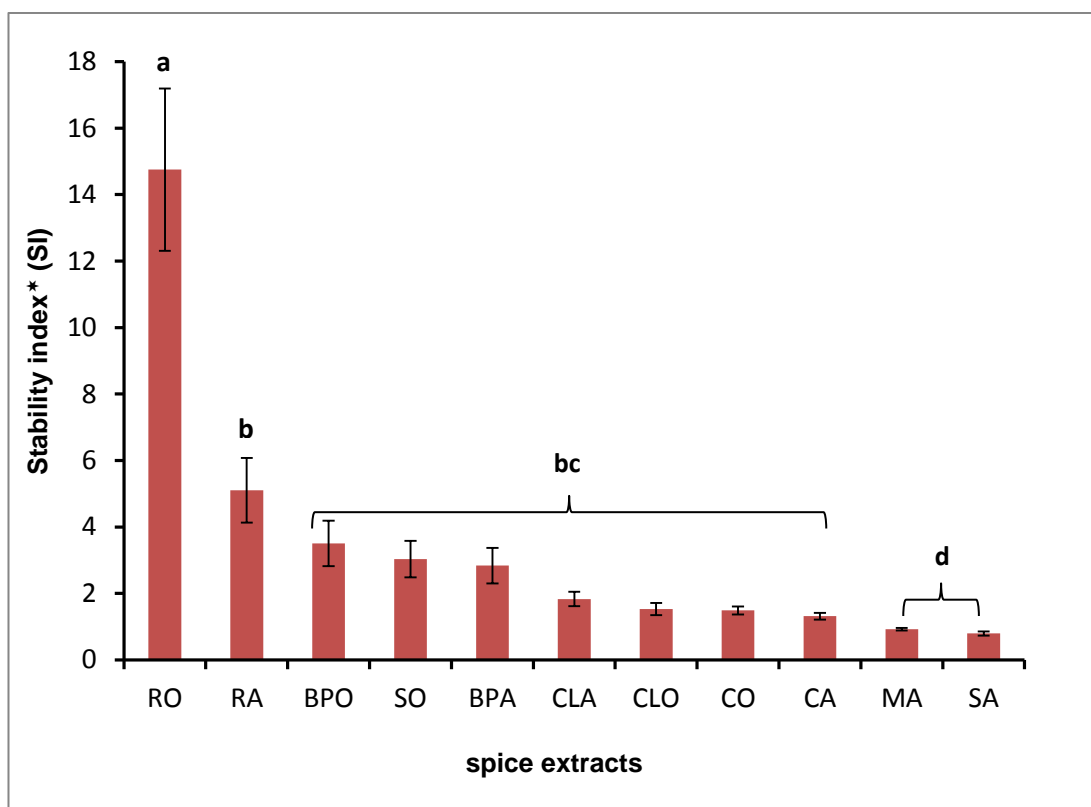


Figure 4.2: Mean \pm SE of stability index of spice extracts.

*Stability index = induction time of the sample/induction time of the control, Error bars are the standard error of the mean (SEM), Bars with different letters are significantly different ($P < 0.05$). Key of the figure as Table 4.1.

According to the mean value of all spice extracts at different concentration used, the antioxidant activity of spice extracts follows this ascending order: RO > RA > BPO = SO = BPA = CLA = CLO = CO = CA > MA = SW.

Spice extracts with highest antioxidant activity ($P < 0.05$) were those obtained from rosemary (RO, RA). However, rosemary oleoresin activity was found to be higher than that of aquaresin ($P < 0.05$).

A moderate antioxidant activity of spice extracts was obtained from black pepper (BPO, BPA), sumac alcoholic extract (SO), clove extracts (CLO, CLA) and cinnamon extracts (CO, CA). The extracts that did not show any antioxidant properties were spearmint (MA) and sumac water extract (SA), which showed activity lower than the control, which could indicate pro-oxidant activity.

There were no significant differences ($P > 0.05$) between oleoresin and aquaresin extracts for black pepper, clove, and cinnamon, while highly significant differences ($P < 0.05$) occur between rosemary and sumac extracts. The stability index of spice extracts was concentration dependent and followed a linear trend with a positive relationship between the concentration and the stability index for all spice extracts, except sumac water extract (SA) which has a negative correlation. The regression equations could be useful to predict the stability index at any concentration (Figure 4.3).

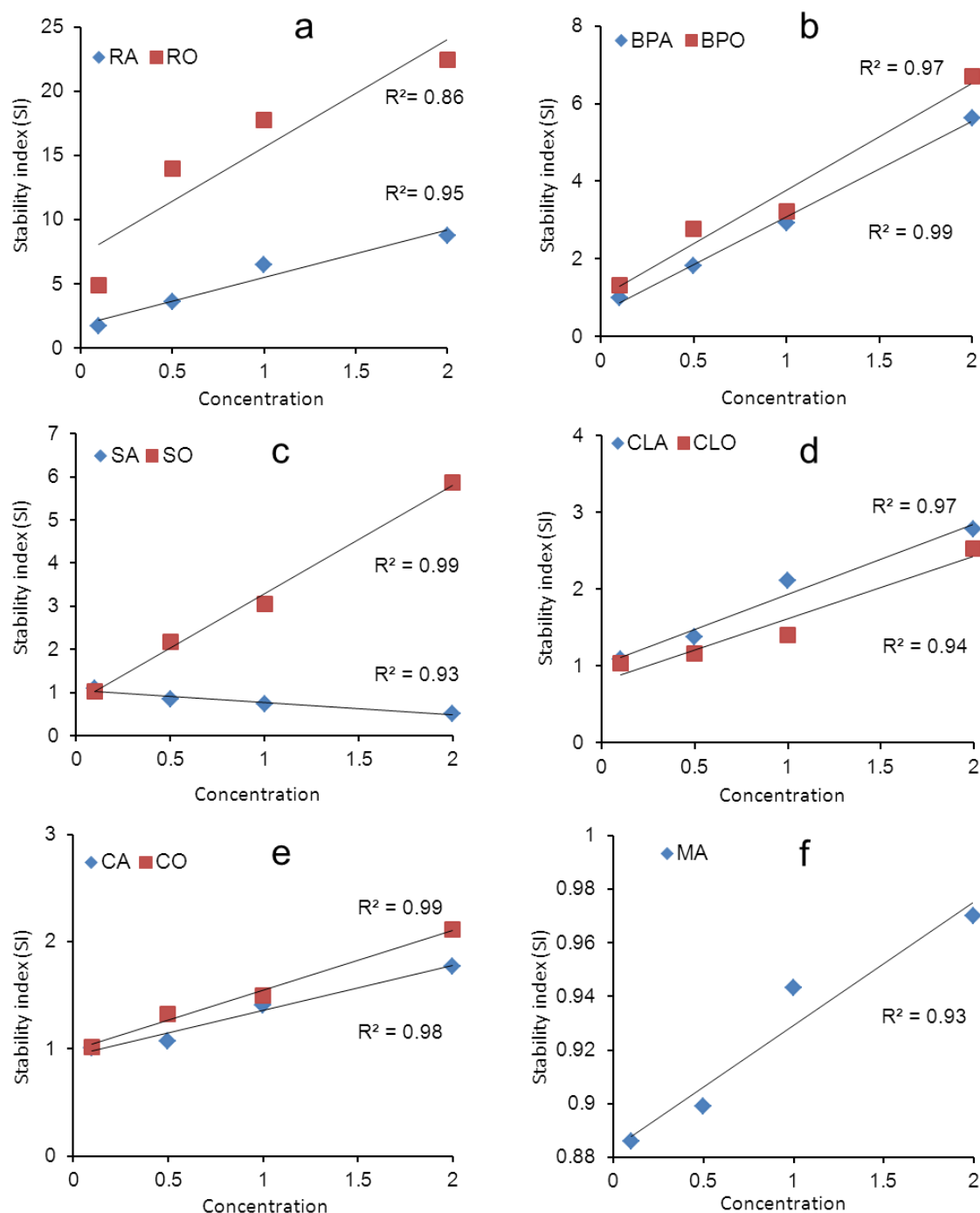


Figure 4.3: The relationship between the stability index of spice extracts and the concentration (w/g) in lamb fat model using Rancimat method, a: rosemary oleoresin and aqueous extract; b: black pepper oleoresin and aqueous extract, c: sumac alcoholic extract and water extracts; d: clove oleoresin and aqueous extract; e: cinnamon oleoresin and aqueous extract; f: spearmint aqueous extract.

4.4 Discussion

4.4.1 Antibacterial activity of spice extracts.

Of the eleven spice extracts tested in this study, there were no significant differences ($P > 0.05$) between oleoresin and aquaresin extracts as determined by the diameter of inhibition zone and the activity unit except for those from cinnamon, sumac, and rosemary, where significant differences ($P < 0.05$) were found. Cinnamon, rosemary oleoresin and sumac alcoholic extract possessed higher antibacterial activity than cinnamon, rosemary and, sumac aquaresin. This may be due to the concentration of the volatile oil (essential oil) being higher in the oleoresin than aquaresin. These extracts had excellent antimicrobial activity despite their relatively low capacity to dissolve in water, in line with current result Lis-Balchin and Deans (1997) and Arora and Kaur (1999).

Cinnamon extracts: cinnamon oleoresin found to be the most inhibitory spice extracts against microorganisms tested Table 4.1. The antimicrobial activity of cinnamon extracts are due to the hydroxyl group in phenolic components present in their composition which represented by cinnamaldehyde and eugenol (Prabuseenivasan et al. 2006, Shan et al. 2007b); others had also recognised cinnamon extracts as strong antibacterial components (Ultee et al. 2002, Agaoglu et al. 2007, Paparella et al. 2008)

Sofia et al. (2007), showed that cinnamon aqueous extracts had a strong antibacterial activity against *E. coli* and *bacillus cereus* at concentrations of 0.5 and 1% (w/v). Ouattara et al. (1997), reported a significant effect of cinnamon essential oil (Cinnamon, ELB 40404) against *Lactobacillus sake* but in this study no inhibition was observed. Differences between results may be related to

many experimental variations such as the specific bacterial strains used, and the methodology employed for the essay.

Clove extracts: Clove (oleoresin and aquaresin) showed antibacterial activity against nine strains of selected bacterial tested with mild inhibitory action of 100-1500 ml⁻¹ AU. Similarly Agaoglu et al. (2007), reported that clove oil exhibited a moderate inhibition effect against *Staph. aureus*. Sofia et al. (2007), reported that clove water extract had strong antibacterial effect against *Bacillus cereus*, *Staph. aureus*, and *E. coli*. The potent antimicrobial activity of clove extracts can be attributed to eugenol which is the most important active substance and the major component (Farag et al. 1989, Blech et al. 1991, Nascimento et al. 2000). Sub-lethal concentrations of eugenol have been found to inhibit production of amylase and proteases by *B. cereus*, cell wall deterioration and a high degree of cell lysis were also noted (Thoroski et al. 1989).

Sumac extracts: sumac hydro alcoholic extract exhibited a strong antibacterial activity against all bacterial strains tested (except *Bacillus subtilis*) with an activity range from 1000-1500 ml⁻¹. These results concurred with those of Nasar-Abbas et al. (2004) who reported that an alcoholic extract of sumac had potent antibacterial activity against most food-borne pathogens and Gram-positive appear to be more sensitive than Gram-negative bacteria.

for sumac aqueous extract the antibacterial activity observed against *Pseudomonas aeruginosa* and *Clostridium perfringens* only with activity unit ranging from 1000-1500 ml⁻¹ respectively; and such results differs from those reported by other studies (Nasar-Abbas and Halkman 2004, Gulmez et al. 2006).

The results obtained by Nasar-Abbas and Halkman (2004), indicated that sumac water extract has a higher antibacterial activity against *Bacillus subtilis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* Gulmez (2006), reported that sumac water extract possessed good antimicrobial activity against psychrotrophic, mesophilic, Enterobacteriaceae, and coliform bacteria in a broiler wing modal.

Rosemary extracts: In the present study, rosemary extracts (oleoresin and aquaresin) showed a moderate antibacterial effect with mean AU of 550 ml⁻¹ and 455 ml⁻¹ respectively. Rosemary oleoresin possessed more activity than rosemary aquaresin which is in agreement with results obtained by Fernández-López et al. (2005), reported that miscible rosemary oil (OR) showed higher activity than water extract (WR) and water and oil extract (OWR), against spoilage bacteria. The phenolic di-terpenoids and camphor are the main compounds of the polar fraction of the rosemary extracts responsible for their antibacterial action (Del Campo et al. 2000). Gram-positive bacteria were found to be more susceptible to rosemary extracts; this is in line with Davidson (1993), reported that the non-polar phenolic components in rosemary are more active against Gram-positive than Gram-negative bacteria. The differences in the antibacterial activity between the rosemary extracts studied (RO, RA), may be either due to the higher volatile oil in RO than RA or the oil extract (RO) is richer in non-polar phenolic compounds.

Spearmint extracts: spearmint aquaresin has a moderate antimicrobial effect against all tested bacteria except for *Pseudomonas aeruginosa*. In line with this results Sofia et al.(2007), found that spearmint aqueous extract has a moderate antibacterial activity against *Staph aureus*, *Bacillus cereus* and *E. coli*. Valero and Salmerón (2003), reported that spearmint aqueous extract has the ability to delay or inhibit the growth of *Bacillus cereus* in carrot broth. however, result from Sagdıç and Özcan (2003), indicated that hydro alcoholic extract of spearmint was ineffective against *Bacillus cereus*, *Staph. aureus*, *E. coli*, and *Salmonella* Typhimurium. The antimicrobial property of spearmint is mainly dependant on the concentration used and the type of microorganism tested.

Black pepper extracts: black pepper extracts (BPO, BPA) had low antimicrobial effect against *Clostridium perfringens* only, perhaps due to oxidation of the two major phenolic compounds, 3,4-dihydroxyphenyl ethanol glucoside A, 3,4-dihydroxy-6-N-ethylamino and benzamide B, which are present in green pepper during the drying process. (Pradhan et al. 1999, Chatterjee et al. 2007). In agreement with the present results, Singh et al.(2005) and Al-Turki (2007). Singh et al.(2005), reported that black pepper had no effect against *Staph. aureus*, *Bacillus subtilis*, *Bacillus cereus*, *E. coli*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa*. While, Al-Turki (2007) added that the black pepper showed the lowest activity among many spice extracts tested. In contrast, Droma and Dean (2000) reported that *piper nigrum* (black pepper) essential oil had antibacterial activity against *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa* and *Staph. Aureus*, this may be due to either different bacterial strain used or different method used for extraction.

4.4.2 Comparison of the Sensitivity of bacterial strains to antimicrobial spice

The results reflected great variation in the sensitivity of bacterial strains against spice extracts, generally Gram-positive bacteria were found to be more sensitive than Gram-negative bacteria (Table 4.1, Figure 4.1). Most studies are in line with current results (Burt 2004, Weerakkody et al. 2010).

Gram-negative bacteria cell wall are surrounded by an outer membrane which is constituted essentially of a lipopolysaccharide (Vaara 1992) that blocks the penetration of hydrophobic oil and avoids the accumulation of essential oils in target cell membranes (Nada et al. 2003).

Due to the large number of diverse groups of chemical compounds present in spice extracts, their mode of action as antibacterial activity is attributable to several mechanisms (Skandamis and Nychas 2001). The hydrophobicity of spice extracts and their essential oil are the main characteristic responsible for their antimicrobial properties. This enables them to penetrate in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and making them more permeable (Sikkema et al. 1994), leading to leakage of cell contents. Extensive damage of cell contents (loss of ions and critical molecules) will lead to death (Lambert et al. 2001, Ultee et al. 2002). In general, spice extracts having the strongest antibacterial activities against spoilage and pathogenic bacteria are those containing a high percentage of phenolic compounds (Juliano et al. 2000, Lambert et al. 2001).

The mechanism of action of those spice extracts is similar to other phenolic compounds and is generally represented by firstly disturbance of the cytoplasmic membrane, secondly disrupting the proton motive force, electron flow and active transport and finally coagulation of the cell contents (Sikkema et al. 1995, Davidson 1997). As reported by Dorman and Deans (2000), the

presence of hydroxyl groups in phenolic compounds play an important role in the antibacterial activity of the spice extracts while, the position of the hydroxyl group on the phenolic ring does not tend to strongly influence the degree of antibacterial activity. As well as, they reported that the non-phenolic components of spice extracts have potential antibacterial effect. Therefore, as it could be expected, spice extracts exhibited different efficacy against tested bacteria.

4.4.3 Antioxidant activity of spice extracts

The antioxidant activity of spice extracts in a lamb fat model were expressed as stability index (SI) and shown in (Figures 4.2 and 4.3).

It was demonstrated that rosemary oleoresin had the best antioxidant activity ($P < 0.05$) among all spice extracts tested at the different concentrations used, followed by rosemary aquaresin. Black pepper oleoresin, sumac alcoholic extract, black pepper aquaresin, clove, and cinnamon had shown intermediate antioxidant effect. While, no antioxidant effect was obtained from spearmint and sumac water extract

Rosemary extracts: The antioxidant properties of rosemary extracts are linked to abundant phenol compounds like carnosic acid and carnosol, which scavenge free radicals and chelate transition metals (Dang et al. 2001, Fernández-López et al. 2005). The activity of carnosic acid results from its lipophilic characteristics (Kilic and Richards 2003b).

previous studies reported that all phenol diterpene compounds such as rosmanol, isomers, epirosmanol and isorosmanol are responsible for the antioxidant activity of rosemary extracts (Nobuji and Reiko 1984, Kenji and Mitsuo 1998). In agreement with current findings (Äzcan 2003, Fernández-

López et al. 2005, Hernández-Hernández et al. 2009), reported that rosemary extracts have higher antioxidant activity among other spice extracts tested. In this study rosemary oleoresin, had higher antioxidant effect ($P < 0.05$) than rosemary aquaresin. This difference may be due to the volatile oil, which is 2-4% in oleoresin and 1-2% in aquaresin.

Black pepper extracts: black pepper showed antioxidant activity with stability index about 3.5 (oleoresin) and 2.8 (aquaresin), which is similar to the results obtained by Agbor et al. (2006), who found that hydrolyzed and non hydrolyzed black pepper had more antioxidant property than white pepper using a free radical scavenging method. Additionally, Gurdip et al. (2004) and Gulcin (2005) reported that both water and ethanol extract black pepper possessed antioxidant activity. The antioxidant activity of black pepper is probably due to the phenol amide compound and acidic fraction of its extract (Kenji and Mitsuo 1998, Gurdip et al. 2004).

Sumac extracts: The antioxidant activity of sumac alcoholic extract was significantly different from sumac water extracts ($P < 0.05$). Sumac alcoholic extract showed remarkable antioxidant activity in the lamb fat model, while sumac water extracts showed pro-oxidant activity. The antioxidant activities of Sumac extracts are due to the anthocyanin and hydrolysable tannin (Hüseyin 2006, Kosar et al. 2007).

Previous studies reported similar results, they indicating that sumac alcoholic extracts display considerable antioxidant activity when used in stabilizing food products. Ozcan and Akgul (1995), reported that sumac methanolic extracts have the ability to stabilize sunflower oil store at 70°C. Moreover, sumac extracts have a significant antioxidant activity for stabilizing peanut oil by

inhibiting the formation of hydro peroxide during the first seven days of addition (Ozcan and Akgul 1995, Ozcan 2003).

Hüseyin (2006), reported that sumac water extracts had a high antioxidant activity in sucuk (Turkish dry fermented sausage) which is in contrast with our results, this might be due to the different method used for extraction, and also different method used for determination of the antioxidant activity of sumac. Current results showed that sumac water extracts display a pro-oxidant activity linked to the sumac water extract component, phenolic autoxidation of hydrolysable tannin which is the main component of sumac water extract is well-known to occur in the presence of oxygen (Barbehenn and Peter Constabel 2011).

Clove extracts: clove extracts demonstrated a moderate antioxidant activity with no differences between clove oleoresin and aquaresin ($P > 0.05$). Similar studies have been also reported that clove extract had significant antioxidant activity, which attributed to phenolic components present in their composition (Lee and Shibamoto 2001, Gülçin et al. 2004, Scherer and Godoy 2009).

The major antioxidant compounds in clove are eugenol and gallic acid (Kramer 1985). Both water and ethanol clove extract have a strong antioxidant activity using methods based on free radical scavenging, superoxide anion radical scavenging, and metal chelating activity (Gülçin et al. 2004).

Cinnamon extracts: Cinnamon extracts appeared to have a moderate antioxidant activity with no significant differences between cinnamon oleoresin and cinnamon aquaresin ($P > 0.05$), as both delay the oxidation of lamb fat more than the control. In line with these results, Shobana and Akhilender (2000) and Lee and Shibamoto (2002). the principal antioxidant components of cinnamon are eugenol and cinnamaldehyd (Lindberg and Bertelsen 1995).

Spearmint extract: spearmint aquaresin did not appear to have any antioxidant activity compared with the control. Shobana and Akhilender (2000), demonstrated that crude water and alcoholic extracts of spearmint leaves had the lowest antioxidant activity compared with other spice extracts (clove, cinnamon, ginger and garlic). However, spearmint paste demonstrated good antioxidant activity in dairy dessert (Sandesh) (Bandyopadhyay et al. 2008). The main antioxidant components of spearmint are phenolic acid and flavonoids (Dimitrios 2006) and the major phenolic acids in the water-soluble spearmint extract are Eriocitrin, luteolin glucoside, rosmarinic acid and caffeic acid (Dorman et al. 2003).

Spice extracts demonstrated antioxidant activity at all concentrations in lamb fat model and the activity was a linear function of concentration. The results agree with observations of previous researchers (Farag et al. 1989, Kanatt et al. 2007), reported a positive linear relationship between the concentration and the antioxidant property of spice extracts.

4.5 Conclusion

Spice extracts of rosemary oleoresin, rosemary aquaresin, cinnamon oleoresin, cinnamon aquaresin, clove oleoresin, clove aquaresin, black pepper oleoresin, black pepper aquaresin, spearmint aquaresin, and sumac aqueous and hydro alcoholic extracts exhibited antibacterial activity *in vitro*. Cinnamon oleoresin and sumac alcoholic extract showed a highest inhibitory effect with a broad-spectrum range against pathogenic and spoilage bacteria.

Different efficacy of spice extracts was observed against specific pathogenic and spoilage bacteria; Gram-positive bacteria are more sensitive than Gram-negative bacteria.

All spice extracts mentioned above showed antioxidant activity except spearmint aquaresin and sumac aqueous extracts, presented pro-oxidant property. Rosemary extract, in particular rosemary oleoresin possessed a high stability index in a lamb fat model, indicating a strong antioxidant effect. Therefore, the application of these potent natural ingredients to high fat meat products (kebabs) could be useful to develop novel meat products with optimum quality and contribute to pathogen control.

Chapter 5

5 Shelf life of cooked doner kebab slices: Effect of temperature, gas storage conditions and spice addition

5.1 Introduction

Lipid oxidation and microbial spoilage are among the most significant quality deterioration factors that signal the end of shelf life in processed perishable food. the oxidative process limiting the quality and acceptability of meat products, which affects lipids, proteins, pigments and vitamins causing discolouration, off-flavour development and loss of nutritive value of meat (Gray et al. 1996, Mataragas et al. 2006, Duong et al. 2008).

Consumers are less likely to accept meat and meat products that have these quality problems. Therefore, the food industry and food processors continue to be directed towards using various technology to control lipid oxidation and to maintain both quality and wholesomeness of the product (Kilic and Richards 2003a).

Several studies have indicated that the lipid oxidation in muscle tissue varies with animal species and depends on different factors which include: the level of polyunsaturated fatty acids present in the particular meat system (Gray et al. 1996, Singh 2000), pre-slaughter effect such as stress (Casimir and Min 2008), post slaughter effect (e.g. temperature of the carcass, pH, disruption of the integrity of muscle membranes by grinding, and cooking) (Gray et al. 1996).

On the other hand, the growth of microorganisms in meat products may cause spoilage, that causes foods to be unacceptable or undesirable for human

consumption due to changes in sensory characteristics (Huis in't Veld 1996, Health Protection Agency 2009b). Some spoiled foods may be safe to eat, but changes in taste, texture, smell, and appearance cause them to be rejected (Brooks et al. 2008) .

Many strategies to retard lipid oxidation and bacterial spoilage in cooked meat products have been examined. These include the use of antioxidants, metal chelating agents, pre slaughter dietary supplement and physical condition such as; controlled atmosphere, vacuum packaging and modified atmosphere packaging (Lou and Yousef 1999, Garcia-Esteban et al. 2004).

Although synthetic additives have been used for retarding lipid oxidation and microbial growth in meat products, over the past few years, the trend is to minimize their use due to the growing concern of consumers about such chemical additives (Chastain et al. 1982). Consequently, the search for natural additives from plant sources including spices and herbs has particularly increased (Bin et al. 2009, Zhang et al. 2009).

Accelerated storage has been a widely used method to determine the shelf life of many industrial products such as food, cosmetic and pharmaceuticals, it is considered as a helpful way in predicting the shelf life of perishable commodities (Corradini and Peleg 2007).

Lamb meat is a very popular ingredient in doner kebab production, which contains high level of fat (20 - 40)% (Anonymous 1995). Thus, doner kebab is likely to be more susceptible to oxidative changes particularly if the fats have been already heat-treated (Kilic and Richards 2003a, Oliveira et al. 2009). There is a lack of information about the shelf life of lamb doner kebab. Therefore, the objectives of this study were to determine the shelf life of vacuum

pack refrigerated doner kebab and to investigate the combined effect of rosemary oleoresin, temperature and packaging types to control lipid oxidation of doner kebab using accelerate shelf life protocols.

5.2 Materials and methods

5.2.1 Experimental design

Two experiments were designed as follows. The first experiment (A) was carried out in order to determine the shelf life of vacuum pack (VP) refrigerated ready to eat doner kebab. The second experiment (B) was performed in order to identify the factors (e.g. temperatures, storage conditions (packaging) and rosemary oleoresin addition (Oleoresin rosemary, NS, 19-06) that influence the shelf life of cooked doner kebab product (this experiment was designed according to an accelerated shelf life determination protocol (Singh 2000). The following is a schematic representation of the two experiments (Table 5.1).

Table 5.1: Schematic representation of the two experiments.

| Experiment | Sample | Treatment | Storage temperature | Storage time |
|------------|---------------------------|---|---------------------|--------------|
| A | Cooked doner kebab slices | VP | 4°C | 11 weeks |
| B | Cooked doner kebab slices | AP VP MAP★ RO and AP RO and VP RO and MAP★ | 20°C and 30°C | 6 days |

VP: vacuum packaging; AP: air packaging; MAP: modified atmosphere packaging; RO: rosemary oleoresin; ★ gas atmospheres composition: (40%CO₂, 30%O₂ and 30%N₂).

5.3 Cooked doner kebab slices

Raw doner kebab as in Section 3.10.1 was prepared and cooked as describe in Section 3.11.

5.4 Antioxidant and application

Rosemary oleoresin, i.e. rosemary oil-miscible extract was provided by Kalsec as in Section 3.1, and the usage level was 0.05 (w/w) according to the suppliers application guide. One part rosemary was mixed with 1.5 part sunflower oil (v/v) as a carrier. Kebab slices were dipped for 1 second and left on a rack to allow the excess to drain.

5.5 Storage condition

In experiment (A), cooked doner kebab slices were vacuum packed individually by using (Multivac MU 61, Germany) and stored at $4\pm1^{\circ}\text{C}$ (Williams Refrigeration and Air-conditioning, installation by general refrigeration, Limited) for 11 weeks (samples were analysed weekly). While, in experiment (B) all three packaging condition treatment samples with spice added were stored in darkness at $20 \pm 1^{\circ}\text{C}$ by using a LMS cooled incubator model 305 (LMS Ltd, Sevenoaks, Kent, UK) and at $30 \pm 1^{\circ}\text{C}$ by using LABMARK-VSL incubator for 6 days, to follow an accelerated shelf life determination protocol (Singh 2000). Samples without treatment were set as controls. Samples were taken as designed before and analysed promptly within six day as follows.

Doner kebab slices analysis

Experiment A: shelf life determination of vacuum pack refrigerated ready to eat doner kebab.

5.6 Biochemical analyses

5.6.1 TBA values

The secondary lipid oxidation products from cooked doner kebab were assessed as in Section 3.13.2.2.

5.6.2 pH value

The pH value recorded on each sampling week (1 - 11 weeks) as in Section 3.13.2.3.

5.6.3 Water activity

Water activity for cooked doner kebab samples was determined weekly for 11 weeks as in 3 Section 3.13.2.4.

5.6.4 Ash

Ash (total mineral or inorganic content) was determined in duplicate according to AOAC, 1995 (Official Methods of Analysis Method 923.03), by adding a known sample weight (~ 500 mg) to a pre-weighed ceramic crucible. The crucibles and samples were then incinerated in a muffle furnace (Carbolite, Sheffield, UK) at 550 °C for 12 hr. After cooling in a dehumidification chamber, the percentage of ash was determined from the sample residue using eq.5.1:

$$\text{Ash (\%)} = \frac{\text{Sample residue (g)} - \text{crucible weight (g)}}{\text{Initial sample weight (g)}} \times 100 \dots \dots \dots (5.1)$$

5.6.5 Fat content determination

Fats content was determined for raw and cooked doner kebab sample in duplicate using the rapid soxhelt extraction method (Tecator Systems, model 1043 and service 1046, Högnäs, Sweden). Kebab samples were weighed (~ 3 g) and placed into a cellulose thimble lightly plugged with cotton wool and inserted into the condensers (raised into the 'rinsing' position) of a SoxTec™ Extraction system (Tecator Systems, model 1043 and service 1046, Högnäs, Sweden). Pre- weighed cups containing 40 ml of petroleum ether were clamped into the condensers and extraction levers moved to the boiling position for 30 min, after which extraction levers were set to the 'rinsing' position for 45 min. The cups containing extracted lipid were then transferred to a fume cupboard, cooled for 30 min and weighed. Total lipid content was determined with eq. 5.2:

$$\text{Total lipid (\%)} = \frac{\text{Cup including Lipid weight (g)} - \text{Cup weight (g)}}{\text{Initial sample weight (g)}} \dots \dots \dots (5.2)$$

5.6.6 Crude protein determination

Determination of the total crude protein in raw and cooked freeze dried doner kebab samples were achieved in triplicate by the Kjeldhal method according to AOAC, 1999a (Official Methods of Analysis Method 988.05), which measures protein from the total nitrogen content of the samples. Total nitrogen was multiplied by a factor of 6.25 to calculate apparent protein content. In brief, ~ 150 mg of dried sample was weighed directly into a micro kjeldhal tube along with one catalyst tablet (3 g K₂SO₄, 105 mg CuSO₄, and 105 mg TiO₂; BDH Ttd., Poole, UK) and 10 ml concentrated sulphuric acid (H₂SO₄) (Specific gravity= 1.84, BDH Ltd. Poole, UK). Digestion was performed with a Gerhardt Kjeldatherm digestion block (Gerhardt Laboratory Instruments, Bonn, Germany) with the following schedule; 100°C for 30 min, 225°C for 45 min (one hour if

samples had particularly high lipid content) and 380°C for 60 min. Once digestion was completed and following a cooling period, the samples were distilled using a Vodapest 40 automatic distillation unit (Gerhardt Laboratory Instruments, Bonn, Germany), the distillate was neutralised with concentrated H₂SO₄ and from the titration value crude protein determined using eq. 5.3:

$$\text{Crude protein (\%)} = \frac{(ST-BT) \times 0.1 \times 14 \times 6.25}{\text{Initial Sample Weight}} \times 100 \dots \dots \dots (5.3)$$

Where; ST is the sample titre (ml); BT is the blank titre (ml); 0.1 is the molarities of the acid; 14 is the relative atomic mass of nitrogen (N); 6.25 is the constant relationship between N and animal protein.

5.6.7 Moisture

Donner kebab cooked and raw samples were weighed and dried at 105°C with a fan assisted oven (Genlab Ltd., UK) until a constant weight was achieved. Percentage moisture was determined using eq. 5.4:

$$\text{Moisture (\%)} = \frac{\text{Wet weight (g)} - \text{Dryweight (g)}}{\text{Sample weight (g)}} \times 100 \dots \dots \dots (5.4)$$

5.6.8 Determination of energy

Energy was determined by using a bomb-calorimeter, under an oxygen atmosphere in a closed vessel which is surrounded by water, under controlled conditions. One gram of freeze dried doner kebab sample was weighed into a crucible and placed into a stainless steel container (decomposition vessel) and filled with oxygen at 435 psi, the sample was electrically ignited through a wire thread inside the decomposition vessel and combusted. During the combustion the core temperature in the crucible is raised up to 1000°C and the pressure rises for a millisecond to approximately 2900 psi; then under these conditions all organic matter was oxidized, the heat created by the combustion process was

transferred into the surrounding water jacket where it was detected; then the internal microprocessor of the bomb calorimeter converts this information in to the energy value. The result was expressed as MJ/Kg of sample.

5.6.9 Salt determination

The Mohr titration method was used to determine the salt content in raw and cooked doner kebab samples by using silver nitrate. Samples 30 gram were ground by a mortar and pestle and 10 gram were transferred together into a conical flask and mixed with 50 ml distilled water, then the samples were boiled for 10 minutes and cooled immediately and filtered through filter Whatman No. 4, in to a 100 ml volumetric flask. The volume of the sample was adjusted to 100ml by adding the distilled water, 5 ml of the filtered sample with three drops of potassium chloride (indicator). Solutions were titrated with 0.1M silver nitrate solution to reach a reddish- brown end point. The percentage of salt (sodium chloride) in sample was determined with eq. 5.5:

$$Salt (\%) = \frac{T \times 0.585 \times 20}{w} \dots \dots \dots (5.5)$$

Where T= mean titrate of 0.1M silver nitrate in ml and W= weight of the original sample used in grams.

5.6.10 Microbiological analysis

Doner kebab samples were taken aseptically for microbiological analysis as in Section 3.13.1.

For the growth model, growth data obtained from TVC and LAB were log transformed and time data sets were fitted to a Baranyi and Roberts model (1994) using MicroFit program version1 (Institute of Food Research, Norwich, UK). This model estimated the kinetic parameter for the initial bacterial density (N_0 CFUml⁻¹) the final bacterial density (N_{max} CFUml⁻¹), the maximum specific growth rate (μ_{max} h⁻¹), the lag time (t-lag h), and the doubling time (t- d h). Additionally, this model calculated an estimate of the error associated with the parameter value such as: the residual sum of squares (RSS), and the root mean square error (RMS).

5.6.11 Sensory analysis

Sensory evaluation followed British Standard Guidelines was performed on cooked doner kebab samples during storage time (1-11weeks) as in Section 3.13.3. The samples were taken out of chilled storage weekly, heated by microwave oven at medium setting for 15 seconds (Brother Hi- Speed Combination Cooker / Japan), and evaluated as in Section 3.13.3.1.

Experiment B study the model of lipid oxidation in cooked doner kebab slices at accelerated shelf life

5.6.12 TBA

TBA was determined as in Section 3.13.2.2.

5.6.13 Determination of stability index during the storage (Rancimat method)

Stability index during storage of each doner kebab sample (0.5g of each sample was added to 2.75 ± 0.01 g of sunflower oil) was determined as in Section 3.13.2.1.

5.7 Statistical Analysis

Data were statistically analysis as in Section 3.14. The regression coefficient was calculated to quantify the relationship between the time and the rancidity represented by (TBA and stability losses). For microbiological data analyses, results were transformed in to logarithm before analyses were reported as mean \pm standard error; also, MicroFit program version1.0 (Institute of Food Research, Norwich, UK) was used.

5.8 Results

Experiment A: shelf life determination of vacuum pack refrigerated ready to eat doner kebab.

5.8.1 Chemical composition

Chemical composition and energy (caloric value) of raw and cooked doner kebab samples are summarized in Table 5.2. There were statically significant differences ($P < 0.05$) between raw and cooked doner kebab products in terms of protein, lipid, ash, moisture, salt and energy.

Table 5.2: Chemical composition in raw and cooked doner kebab sample.

| Composite | Product | |
|------------------------|--------------------------|--------------------------|
| | Raw doner kebab | Cooked doner kebab |
| Protein(g/100g Meat) | 12.10±0.069 ^a | 22.36±0.584 ^b |
| Lipid (g/100g meat) | 28.88±0.077 ^a | 19.41±0.07 ^b |
| Ash (g/100g meat) | 2.11±0.009 ^a | 3.36±0.659 ^b |
| Moisture (g/100g meat) | 48.73±0.805 ^a | 43.35±0.012 ^b |
| Salt g/100g | 0.51 ^a | 1.84 ^b |
| Energy (MJ/Kg) | 29.44±0.082 ^a | 26.32±0.012 ^b |

a, b: Means with the different letters in a row are statistically different ($P < 0.05$), Result expressed as a Mean ± standard error, n=3

5.8.2 Water activity

The changes in the water activity of cooked kebab sample over time are illustrated in Figure 5.1. Water activity slightly increased with storage time ($P > 0.05$), ranged from a value of 0.942 at week 0 to a value of 0.948 at week 11.

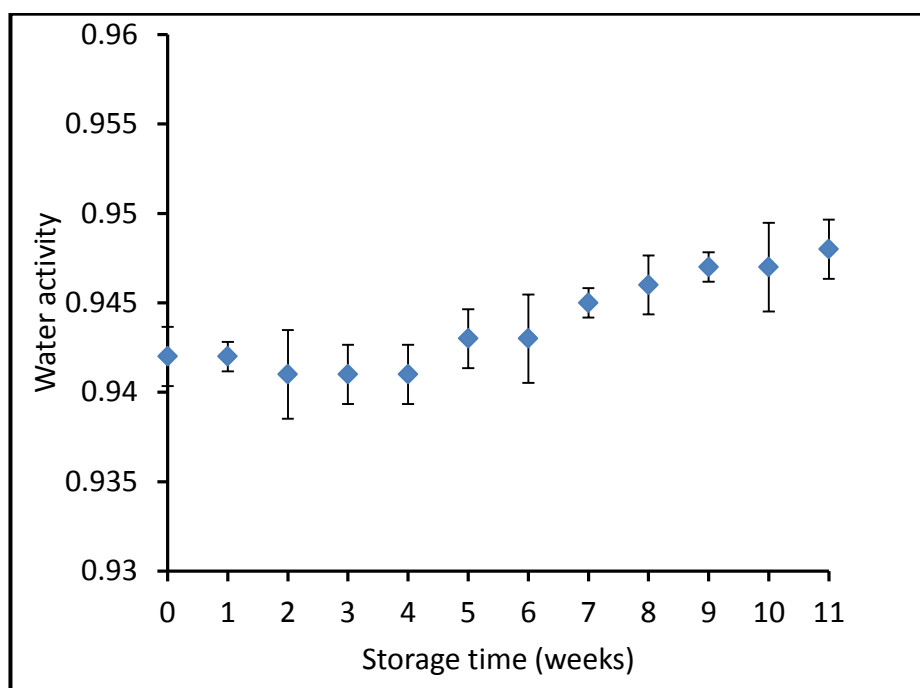


Figure 5.1: Effect of storage time on water activity of refrigerated (4°C) vacuum pack doner kebab slices. Result represented as mean \pm standard error (n=3).

5.8.3 PH value

Changes in pH values of cooked doner kebab samples during storage under vacuum packaging conditions are shown in Figure 5.2. The pH value of cooked doner kebab product remained nearly stable with storage time, the initial pH of cooked doner kebab product was 6.07 and the final value was 6.12 ($P > 0.05$).

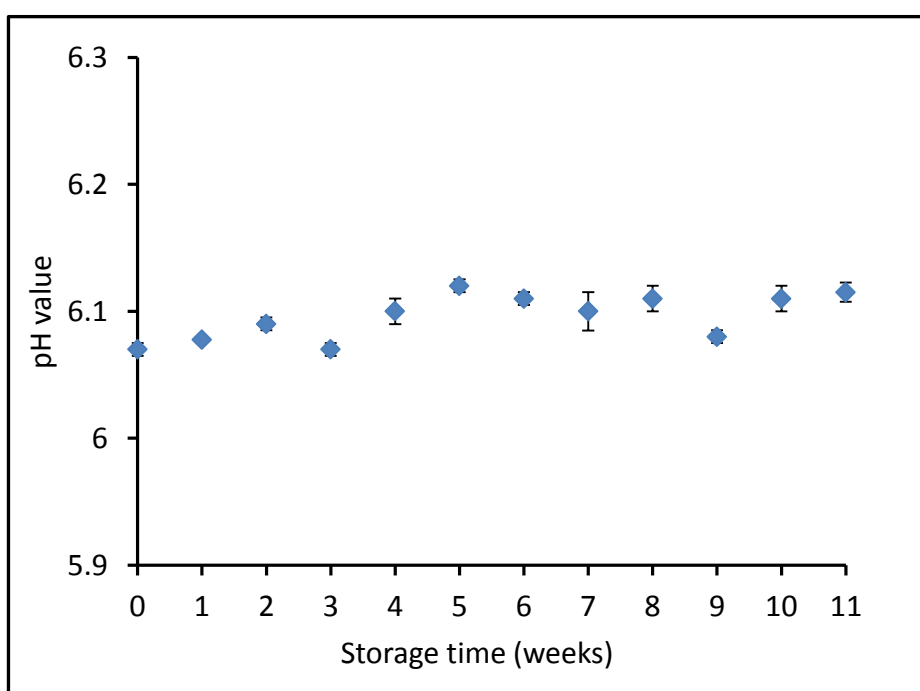


Figure 5.2: pH values of vacuum pack doner kebab slices during storage time at 4°C. Result represented as mean \pm standard error (n=3).

5.8.4 TBA value

The change in malonaldehyde concentration with respect to storage time is illustrated in Figure 5.3. TBA values of the samples increased linearly ($R^2=0.98$) with time. The statistical analysis showed that storage time had a significant effect ($P < 0.05$) on TBA values.

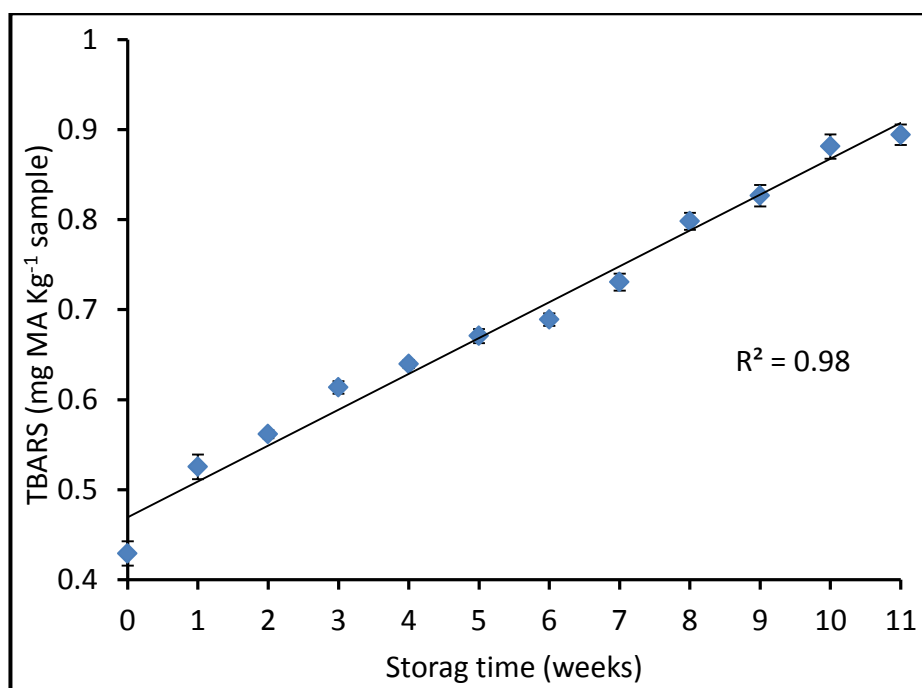


Figure 5.3: Rancidity (TBA) evaluations of vacuum pack ready to eat doner kebab slice during storage time at 4°C. Result represented as mean \pm standard error ($n=3$).

5.8.5 Microbiological analysis

Enterobacteriaceae, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, *Listeria monocytogenes*, and moulds and yeasts, were not detected in any cooked doner kebab slices, regardless of storage time.

LAB and TVC were detected in doner kebab slices, the growth of TVC increased exponentially with storage time, the initial bacterial count was 4.1 (log CFU /g) while the final was 6.38 (log CFU /g) as shown in Figure 5.4.

For LAB, the initial population was found to be 3.26 (log CFU /g) and the final bacterial count was 3.53 (log CFU /g) as shown in Figure 5.5.

The predictive growth curve of microbial population for TVC and LAB was determined using the Baranyi and Roberts model and the MicroFit programme (Table 5.3).

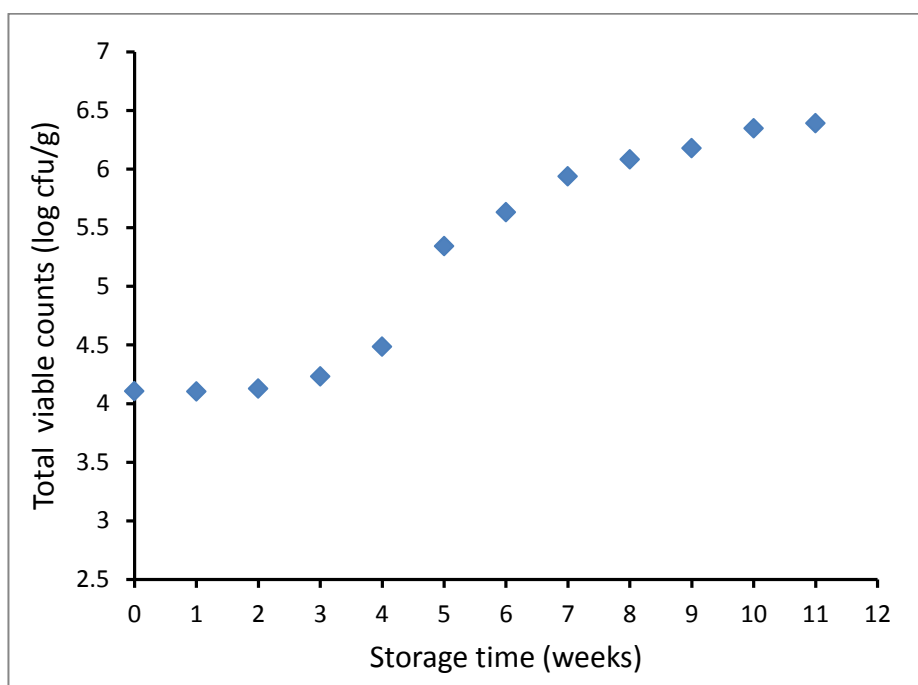


Figure 5.4: Changes in total viable count of vacuum packaging cooked doner kebab slices during storage time at 4°C. Result represented as mean \pm standard error (n=3).

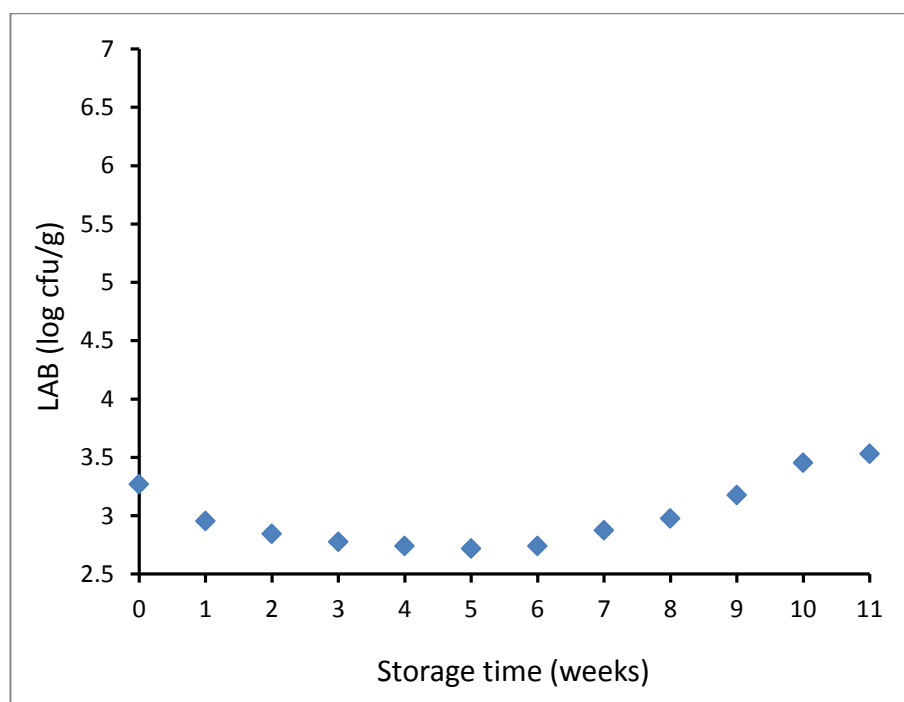


Figure 5.5: Changes in lactic acid bacteria of vacuum packaging cooked doner kebab slices during storage time at 4°C. Result represented as mean \pm standard error (n=3).

Pearson correlation between lipid oxidation (TBARS) and bacterial growth (TVC) in doner kebab sample showed a significant ($P < 0.05$) positive association with $r = 0.90$ Figure 5.6.

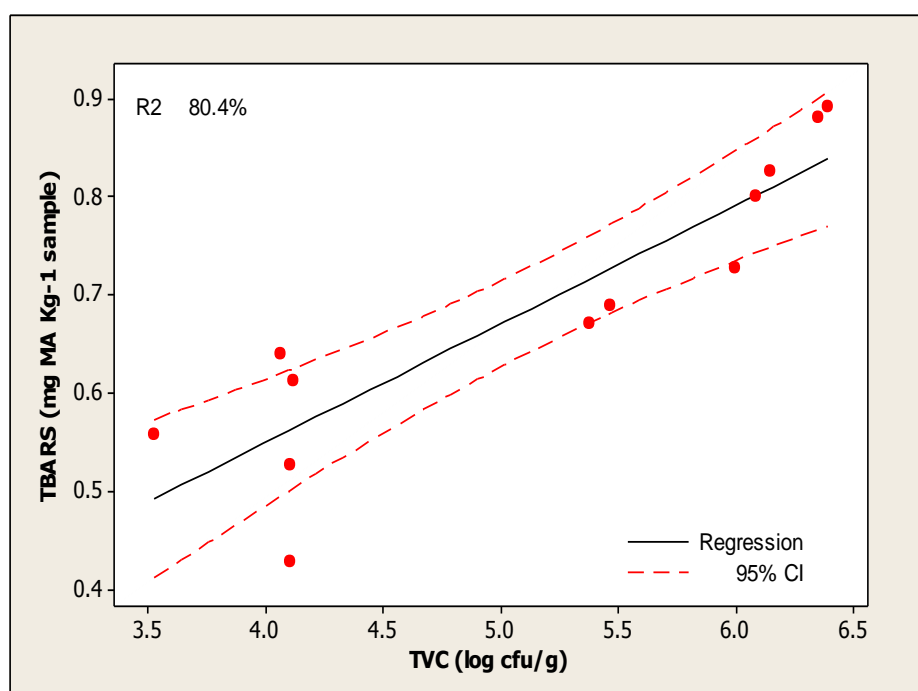


Figure 5.6: Correlation between lipid oxidation (TBARS) and bacterial growth (TVC) of vacuum packaging cooked doner kebab slices during storage time at 4°C.

Table 5.3: Growth model for TVC and LAB of cooked doner kebab slices stored at 4°C at storage period (0-11 weeks) by applying Baranyi and Roberts equation.

| Equation parameter | Bacterial group | | |
|-----------------------|-----------------|-----------|-----------|
| | TVC | LAB | |
| | 0-11 week | 0-4 week | 4-11 week |
| N_0 | 3.91±0.15 | 3.25±0.09 | 2.75±0.03 |
| N_{max} | 6.24±0.14 | 2.65±0.80 | 3.62±0.08 |
| μ_{max} | 1.81±0.54 | 0.47±3.15 | 1.20±0.24 |
| t-lag | 3.65±0.62 | 1.29±3.67 | 8.08±0.2 |
| t-d | 0.38±0.21 | 1.46±4.60 | 0.58±0.14 |
| RSS | 0.05 | 0.01 | 0.01 |
| RMS | 0.02 | 0.04 | 0.03 |

N_0 : the initial bacterial density, N_{max} : the final bacterial density, μ_{max} : the maximum specific growth rate (h^{-1}), t-lag: the lag time, t- d: the doubling time, RSS: the residual sum of squares, RMS: the root mean square error.

5.8.6 Sensory evaluation

From the six sensory attributes evaluated, only one attribute (aroma) showed significant change due to storage time ($P < 0.05$). The products were stable for six weeks, with some differences becoming apparent from week 7 until the end of the experiment in aroma score. While, for texture, colour, overall appearance and overall acceptance, no significant change ($P > 0.05$) were detected during the storage time; although all the score were decreased. The scores were all in the well-accepted region for the panel evaluation Table 5.4

Table 5.4: Evaluation of sensory scores for aroma, texture, colour, overall appearance and overall acceptance of doner kebab slices stored under vacuum packaging at 4°C for 11 weeks.

| Storage time (weeks) | Attribute* | | | | |
|----------------------|------------------------|-----------|-----------|--------------------|--------------------|
| | Aroma | Texture | Colour | Overall appearance | Overall acceptance |
| 0 | 6.50±0.34 ^a | 4.83±0.48 | 5.50±0.56 | 5.33±0.55 | 4.83±0.91 |
| 1 | 6.00±0.33 ^a | 4.50±0.75 | 5.30±0.56 | 5.20±0.50 | 5.00±0.50 |
| 2 | 6.29±0.17 ^a | 5.57±0.56 | 5.57±0.50 | 5.57±0.56 | 5.71±0.42 |
| 3 | 6.17±0.31 ^a | 5.33±0.72 | 5.50±0.72 | 5.33±0.56 | 5.50±0.56 |
| 4 | 6.00±0.37 ^a | 4.67±0.72 | 5.50±0.56 | 5.00±0.82 | 5.00±0.82 |
| 5 | 5.83±0.31 ^a | 5.17±0.48 | 5.17±0.48 | 5.00±0.63 | 5.17±0.71 |
| 6 | 5.83±0.31 ^a | 5.33±0.56 | 4.50±0.34 | 5.00±0.45 | 5.00±0.45 |
| 7 | 5.33±0.21 ^b | 4.83±0.40 | 4.50±0.50 | 4.17±0.40 | 4.33±0.33 |
| 8 | 5.57±0.22 ^b | 5.00±0.66 | 4.71±0.58 | 4.57±0.48 | 4.57±0.48 |
| 9 | 5.50±0.43 ^b | 4.67±0.62 | 4.67±0.62 | 4.50±0.56 | 4.50±0.67 |
| 10 | 5.50±0.43 ^b | 4.50±0.67 | 4.67±0.62 | 4.33±0.67 | 4.33±0.76 |
| 11 | 5.50±0.43 ^b | 4.67±0.72 | 4.83±0.71 | 4.67±0.81 | 4.33±0.76 |

Values are given as mean (n=6) ± standard error of six panellist scores, *Based on 7 point descriptive scale. ^{a-b}, means followed by different letters within the row are significantly different ($P < 0.05$).

Experiment B- study of lipid oxidation in cooked doner kebab slices at accelerated shelf life on a model system.

5.8.7 Lipid oxidation of doner kebab

5.8.7.1 TBA Evaluation (Rancidity)

The effect of the temperature, spice and type of packaging on lipid oxidation of cooked doner kebab during storage is shown in Figures 5.7 - 5.9. The analysis of variance for the TBARS data indicates that the TBA values were significantly affected ($P < 0.05$) by both the storage time, temperature and packaging method. Moreover, the samples with spices showed lower TBA values than control samples, which mean that rosemary oleoresin (19-06) has a potent effect in retarding lipid oxidation. At the end of storage times MAP showed significantly higher TBA values ($P < 0.05$) compared with AP and VP.

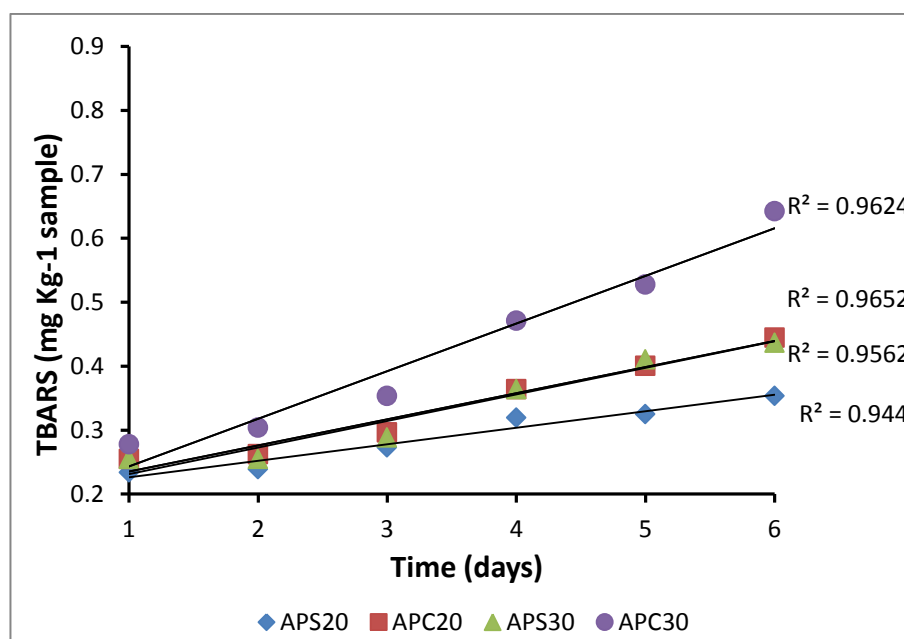


Figure 5.7: Rancidity (TBA) evaluation in air packaging cooked doner kebab slicesa with different storage temperatures (20°C, 30°C) during storage time. a: Doner kebab slices: APS: air packaging doner kebab slice treated with spice extract (rosemary oleoresin), APC: air packaging doner kebab slice without spice (control).

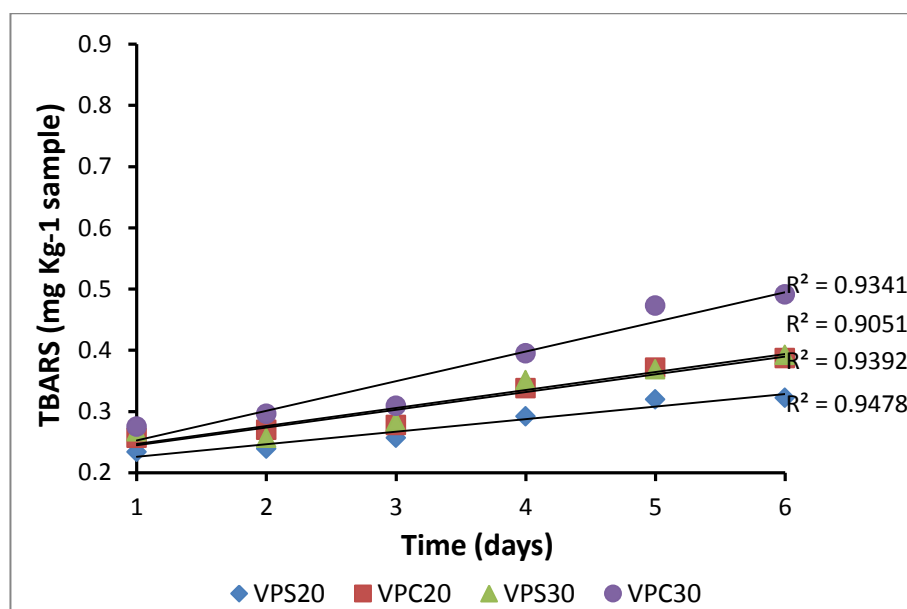


Figure 5.8: Rancidity (TBA) evaluation in vacuum packaging cooked doner kebab slicesa with different storage temperature (20°C, 30°C), during storage time. Doner kebab slices: VPS: vacuum packaging doner kebab slice treated with spice extract (rosemary oleoresin), VPC: vacuum packaging doner kebab slice without spice (control).

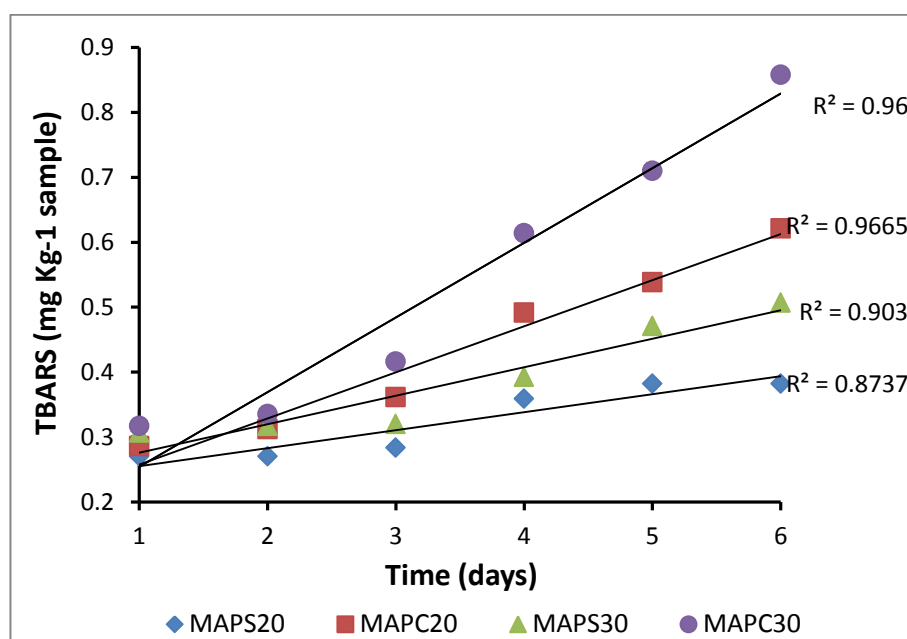


Figure 5.9: Rancidity (TBA) evaluation in modified atmosphere packaging (MAP) cooked doner kebab slicesa with different storage temperature 20°C, 30°C during storage time. Doner kebab slices: MAPS: modified atmosphere packaging doner kebab slice treated with spice extract (rosemary oleoresin), MAPC: modified atmosphere packaging doner kebab slice without spice (control).

5.8.7.2 Stability losses

The Rancimat method was used to determine the amount of oxidation (stability loss index) of cooked doner kebab treated with rosemary oleoresin and stored in different temperature with different packaging methods used (Figures 5.10 - 5.12). The stability loss index increased not only with the increase of storage times but also with increasing temperatures (the stability loss index at 30°C was higher than at 20°C) with significant differences ($P < 0.05$). Stability loss index linearly increased as storage time progress, the results showed a high regression coefficient (Table 5.6). Additionally, spice addition and type of packaging played an important role in extending the shelf life of the products with significant differences ($P < 0.05$) between products. Adding spice (rosemary oleoresin), retarded the oxidation for all packaging methods used, when compared with the control samples. Regarding to the effect of packaging types, VP was found to be the most effective packaging type used, extending the shelf life of the product by minimizing the stability losses, followed by AP and MAP respectively.

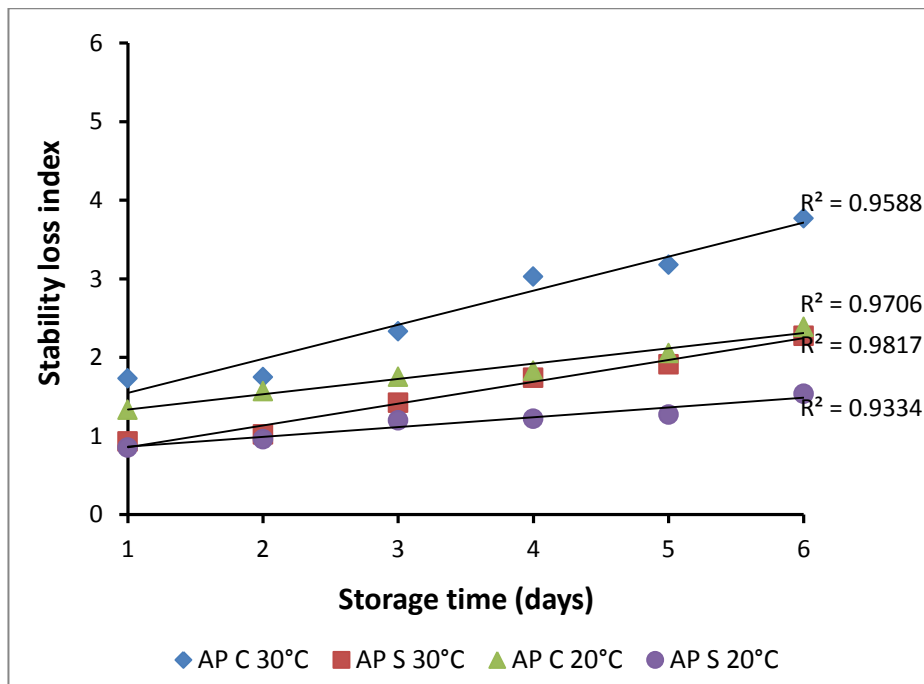


Figure 5.10: Stability losses of air packed doner kebab slices during storage time at different temperatures. Key of the treatments is listed under table 5.5.

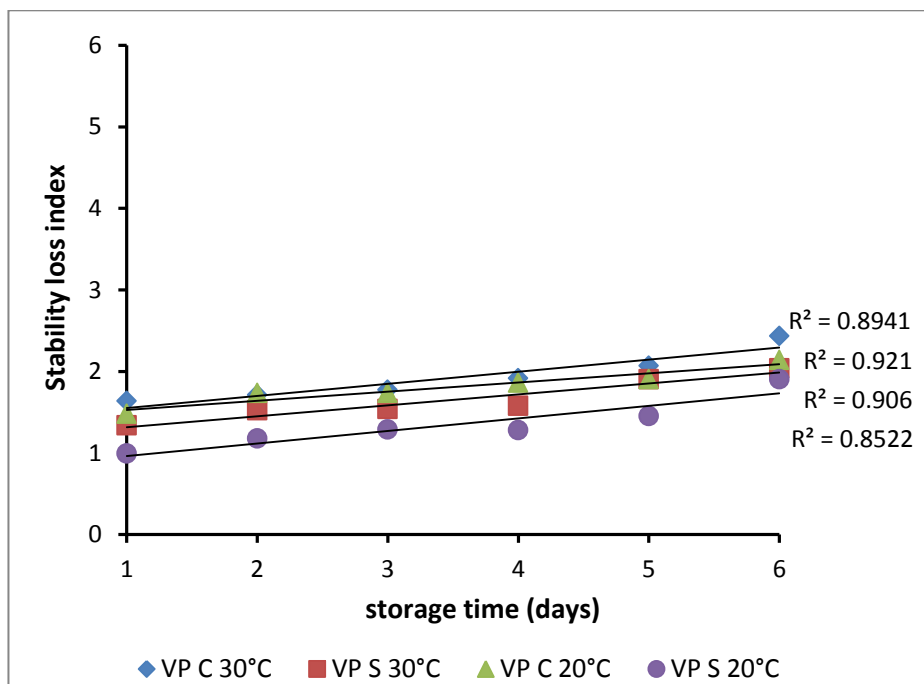


Figure 5.11: Stability losses of vacuum-packed doner kebab slices during storage time at different temperatures. Key of the treatment are listed under table 5.5

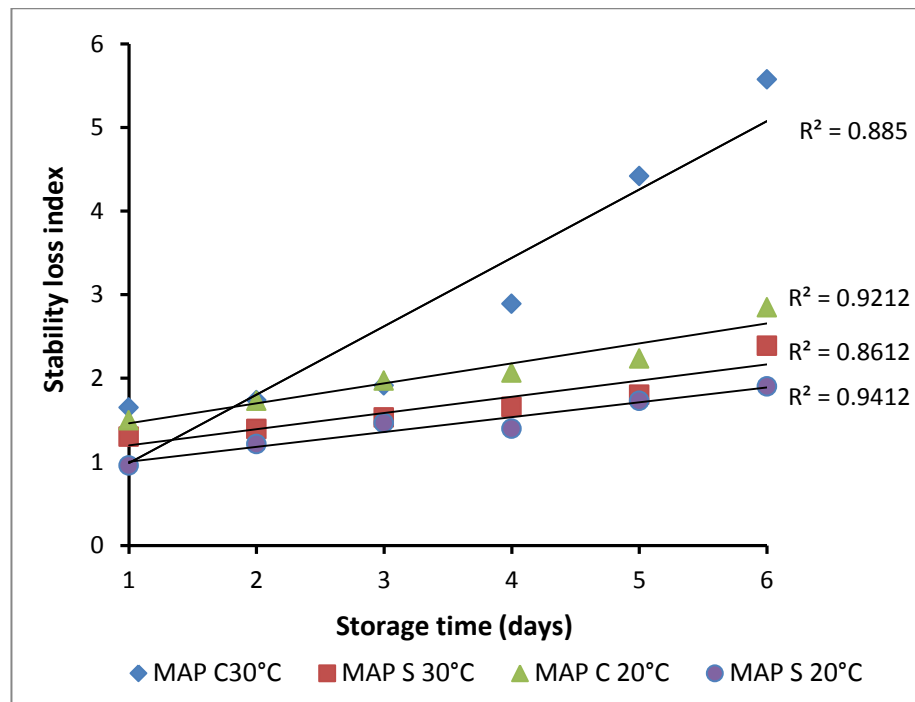


Figure 5.12: Stability losses of modified atmosphere packed doner kebab slices during storage time at different temperatures. Key of the treatment are listed under table 5.5

Table 5.5: The relation expressed as regression of stability losses of doner kebab slices treated with different condition on days of storage.

| Storage Temperature | Treatment ¹ | Means of Stability losses * | Regression | | |
|---------------------|------------------------|-----------------------------|------------|------|------|
| | | | a* | b** | r*** |
| 20°C | APC | 1.82 ^a | 0.19 | 1.14 | 0.97 |
| | APS | 1.18 ^b | 0.74 | 0.13 | 0.93 |
| | VPC | 1.81 ^{ac} | 1.41 | 0.11 | 0.92 |
| | VPS | 1.35 ^{bd} | 0.80 | 0.15 | 0.85 |
| | MAPC | 2.06 ^{ae} | 1.22 | 0.24 | 0.92 |
| | MAPS | 1.45 ^{fd} | 0.18 | 0.13 | 0.94 |
| 30°C | APC | 2.63 ^g | 1.11 | 0.43 | 0.96 |
| | APS | 1.55 ^h | 0.58 | 0.28 | 0.98 |
| | VPC | 1.92 ⁱ | 1.4 | 0.15 | 0.89 |
| | VPS | 1.65 ^{jh} | 1.18 | 0.13 | 0.91 |
| | MAPC | 3.03 ^{gk} | 0.17 | 0.82 | 0.86 |
| | MAPS | 1.68 ^{lhj} | 1.00 | 0.19 | 0.86 |

Stability losses = induction time of the sample/ induction time of the control; ^{a-l} Means within a column with different letters are significantly different ($P < 0.05$); a*: intercept, b*: regression, r***: correlation coefficient; ¹APC: air packaging doner kebab slice without spice adding (control), APS: air packaging doner kebab slice treated with spice extract (rosemary oleoresin); VPC: vacuum packaging doner kebab slice without spice adding (control), VPS: vacuum packaging doner kebab slice treated with spice extract (rosemary oleoresin); MAPC: modified atmosphere packaging doner kebab slice without spice adding (control), MAPS: modified atmosphere packaging doner kebab slice treated with spice extract (rosemary oleoresin).

Table 5.6: The reaction rate of cooked doner kebab slices store at 20°C, and 30°C which calculated by zero order rate equation.

| Storage condition | Qj | Qi | k | Equation Y= A + Bx |
|-------------------|-------|-------|-------|-----------------------|
| APC30 | 1.865 | 2.479 | 0.102 | y = 0.43x + 1.11 |
| APC20 | 1.402 | 1.607 | 0.034 | y = 0.19x + 1.14 |
| APS30 | 0.838 | 1.211 | 0.062 | y = 0.28x + 0.58 |
| APS20 | 0.845 | 0.930 | 0.014 | y = 0.13x + 0.74 |
| VPC30 | 1.645 | 1.764 | 0.020 | y = 0.15x + 1.40 |
| VPC20 | 1.580 | 1.654 | 0.012 | y = 0.11x + 1.41 |
| VPS30 | 1.362 | 1.455 | 0.016 | y = 0.13x + 1.18 |
| VPS20 | 0.962 | 1.102 | 0.023 | y = 0.15x + 0.81 |
| MAPC30 | 1.519 | 4.729 | 0.535 | y = 0.82x + 0.17 |
| MAPC20 | 1.580 | 1.903 | 0.054 | y = 0.24x + 1.22 |
| MAPS30 | 1.254 | 1.465 | 0.035 | y = 0.19x + 1.00 |
| MAPS20 | 0.993 | 1.162 | 0.028 | y = 0.18x + 0.82 |

Qj: the initial value of the quality losses; Qi: the quality loss at the end of shelf life; k: reaction rate. Key of the table are listed under table 5.5

5.9 Discussion

Experiment A: shelf life determination of vacuum packed refrigerated ready to eat doner kebab.

5.9.1 *Chemical composition*

The chemical composition of lamb doner kebab is given in Table 5.2. In general, the protein content, ash and salt of the cooked samples were higher than that of the raw samples ($P < 0.05$). On the other hand, lipid, moisture and energy of cooked doner kebab products showed significantly decrease value comparing with raw doner kebab sample ($P < 0.05$). Lower fat contain in the cooked sample was expected and that due to lose some fat by melting and draining with heating. Additionally there was an 11% moisture reduction in cooked doner kebab. These results concur with those of Kayisoglu et al.(2003) who reported that the cooking process increased the protein, ash and salt contain of cooked doner kebab, whereas decrease the moisture and fat contain in the product. Additionally, Seeger et al.(1986) found that 80% of cooked doner had less than 35.3% fat and more than 12.2% protein, while the moisture varied between 14.5–63.8% which is similar to the values found in this study (Table 5.2).

5.9.1.1 **Water activity**

Water activity of refrigerated vacuum packed cooked doner kebab slices were remain constant as storage time progress ($P > 0.05$) and that may be due to the type of packaging (vacuum packaging) and also because the product was kept in dark, cold place. This finding is in agreement with Steele (2004), reported that water activity in muscle food was generally decreased as storage time progress.

5.9.2 pH value

There were no significant changes ($P > 0.05$) in pH values of vacuum-packed refrigerated doner kebab samples during storage (Figure 5.2). Similarly, Viuda-Martos et al. (2010a) found that storage conditions (air pack, VP and MAP) had no significant influence on pH values of mortadella stored at 4°C during its shelf life. Whereas, Gurunathan et al.(2009) reported that the pH value meat pate was mainly affected by the storage time. According to Giatrakou et al.(2010), The pH values of untreated and treated aerobically pack ready to cooked doner kebab (RTC) samples increased during the first 4 days of storage, whereas beyond this period in time, and up to day-10 of storage, decreased.

Increases in pH during the storage period resulting from the accumulation of the basic metabolites due to protein degradation caused by microbes (Bell and Shelef 1978). while, decreases in pH during storage is due to fermentation of different levels of carbohydrate which mainly depend on the genus, species and growth condition of bacteria (Borch et al. 1991).

These variations from our results were mainly due to type of meat product or type of packaging method used. The pH value of doner kebab samples ranged from 6.07- 6.12 Figure 5.2. This finding is similar to Nemati et al. (2008), reported that the pH of doner kebab range from 5.99–6.45, while Kayisoglu et al. (2003) found pH values ranging from 5.94- 6.04 in cooked beef doner kebabs marketed in Turkey.

5.9.3 TBA value

The effect of the vacuum packaging on lipid oxidation of cooked doner kebab during storage at 4°C is shown in Figure 5.3. The analysis of variance for the TBARS data indicates that the TBA value was significantly changed from 0.43 – 0.89 ($P < 0.05$) as storage time progressed.

An extensive number of studies had investigated the influence of cooking and refrigeration storage on overall meat quality, and specially the development of rancidity during storage (Asghar et al. 1988, Gray et al. 1996, Fasseas et al. 2008, Gurunathan et al. 2009). This oxidation which occurs in meat products is due to mincing, mixing and cooking processes which liberate hem iron from myoglobin, leading to accelerating lipid oxidation, which subsequently increases lipid hydroperoxides (Kanner 1994b). However, Chen et al (2008) Sato and Hegarty (2005) indicated that iron was released from heme pigments during cooking and that led to increases in non-heme iron which was the active catalyst in cooked meats products responsible for lipid oxidation.

Gray et al. (1996) had another explanation, that lipid oxidation in muscle systems is initiated at the membrane level in the intracellular phospholipids' fractions. According to Tarladgis et al.(1960) and Jayasingh et al.(2002) a limit of 1 mg malonaldehyde/kg meats has been suggested for sensory perceived rancidity because there is no legal threshold for secondary oxidation products, such as TBA. In current study, the TBA value at the end of the experiment was not achieving this threshold limit (1 mg malonaldehyde /kg doner kebab).

5.9.4 Microbiological analysis

Reduction in the initial microbial load could be attributed not only to the direct destruction of bacteria by heating (Lawrie, 1998) due to the metabolic injury of the microorganisms (Leistner and Gould 2002).

The TVC count increased significantly ($P < 0.05$) during storage Figure 5.4. The increase in microbial counts was attributed to the available nutrients, conducive a_w , pH and the packaging condition (Leistner et al. 1981).

For LAB, two types of growth were observed Figure 5.5. The bacterial population decreased from $(3.27 - 2.74) \log \text{CFUg}^{-1}$ in week 0 and 4 respectively. Then the bacterial load increased to reach $3.53 \log \text{CFUg}^{-1}$ at week 11. The explanation of that, is due to the packaging method (vacuum packaging), which prevents the growth of aerobic bacteria, as the residual oxygen was consumed by microbial metabolism (Waites 1988). At week 0 all aerobic and anaerobic bacteria were growth, but once all oxygen was consumed, only the anaerobic LAB would growth and increase as the sample storage time progressed. The growth of LAB is stimulated by the CO_2 enriched atmosphere, which is developed in the vacuum package. Consequently, the bacterial counts increased at the end of storage period, as suggested by Waites (1988). According to Health Protection Agency (2009b) the aerobic colony count (TVC) level for ready to eat meat products $<10^6 \log \text{CFU g}^{-1}$ satisfactory, $(10^6 - < 10^8) \log \text{CFU g}^{-1}$ Borderline, and $\geq 10^8 \log \text{CFU g}^{-1}$ unsatisfactory. However, in this study, TVC and LAB were not achieved the unsatisfactory levels, the bacterial counts at the end of the experiment were $(6.4 \text{ and } 3.5) \log \text{CFUg}^{-1}$ respectively and yeast not detected during the storage time.

Similar to our result those of (Rodriguez-Perez et al. 2003) , indicated that lactic acid and psychrotrophic bacterial counts did not achieve the level of spoilage for vacuum packed sliced cooked chicken breast due to both type of packing and cold storage that increased the shelf-life of the product (Rodriguez-Perez et al. 2003). The increase in microbial load in doner kebab possibly caused an increase in oxidative changes, indicating a positive correlation between microbial load and TBA value (Figure 5.6).

However, Enterobacteriaceae, *E. coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Listeria monocytogenes*, yeast, and mould were not detected at any time during storage. The absence of these microorganisms indicated that the doner kebab product was prepared following safe practices resembling commercial aseptic conditions, with a heat treatment that guaranteed pasteurization, which effectively inactivating vegetative cells, and then it was stored under conditions that would limit the growth of bacteria (Fernández-López et al. 2005). The results obtained from MicroFit modelling for TVC and LAB have shown that the model was reliable for obtaining accurate estimates of microbial growth kinetics Table 5.3.

The results shown that for TVC and LAB, Baranyi and Roberts model gave marginally lower estimates for N_0 , and N_{max} and the residual sum of square (RSS) and root mean square error values (RMS) were small (0.05 - 0.01) and could be assumed that these values represented mostly analytical error due to the viable count procedure. The root means square error (RMS) values provide a measure of the goodness of fit for a model to the data used to produce it. The lower value of RMS, the closer is the fit of the model to the data (Banja 2010).

5.9.5 Sensory evaluation

From the five sensory attributes evaluated, only aroma score was significant decreases during storage ($P < 0.05$), while for texture, colour, overall appearance and overall acceptance no differences were obtained ($P > 0.05$). The decline in the aroma score might be attributed to lipid oxidation (Lai et al. 1995, Fernández et al. 1997a), increased liberation of free fatty acid (Branen 1979), and also due to increased microbial load (Santamaria et al. 1992). It can be noticed that the decline in aroma scores are consistent with increases in TBARS values. The primary initial products of lipid oxidation (hydroperoxides) are essentially odourless, but will decompose to a variety of volatile and non-volatile secondary products (Mottram 1994). Drumm and Spanier (1991) explained the relationship between classes of volatile compounds and their flavour thresholds. Additionally, they reported that the carbonyl compounds have the greatest impact on flavour leading to their low flavour thresholds in comparison with the hydrocarbons, substituted furans, and alcohols. These investigators concluded that aldehydes are major contributors to the increase in undesirable flavour in meats because of their rate of formation during lipid oxidation and low flavour threshold.

Experiment B- study the model of lipid oxidation in cooked doner kebab slices at accelerated shelf life

5.9.6 Lipid oxidation of cooked doner kebab

Some physiochemical indices, including TBA and stability losses have been reported to have an important role in monitoring quality and shelf life of meat and meat products (Kristinsson et al. 2001). Therefore TBA and Rancimat methods were used to determine the lipid oxidation of doner kebab.

5.9.6.1 TBA Evaluation (Rancidity)

Lipid oxidation, expressed by TBA values is shown in (Figure 5.8- 5.10) and (Table 4.5), which illustrate the effects of time, packaging, spice adding and temperature for cooked doner kebab. The TBA values were positively correlated with the storage ($P < 0.05$). In meat product lipid oxidation occurred due to the presence of sufficient quantities of polyunsaturated fatty acids in the phospholipids fraction of the intramuscular lipids (Varnam and Sutherland 1995).

Storage in vacuum, modified atmosphere ($O_2/CO_2/N_2$), and air, had a major influence on the oxidative stability of doner kebab slices (Figures 4.8- 4.10). The kebab slices stored in vacuum packaging (VP) were found to have little lipid oxidation ($P < 0.05$) followed by aerobic packaging (AP) and modified atmosphere packaging (MAP) respectively. The rate of oxidation (TBA values) was dependent on the level of O_2 in the bag. Restricted access to air has previously been reported to limit oxidation processes (Jantawat and Dawson 1980, Barbut et al. 1990, Pettersen et al. 2004) which corresponded well with this results.

Many researchers have reported the effectiveness of packaging methods on lipid oxidation in meat and meat products (Sánchez-Escalante et al. 2001, Cayuela et al. 2004). as found in this study, Smiddy et al.(2002) reported that less lipid oxidation in VP than MAP cooked chicken patties stored at refrigeration temperature. Additionally, Lund et al. (2007) indicated that a high level of oxygen in the package increased lipid oxidation of meat product during storage. according to Ahn et al. (1992), VP effectively reduce the TBA values of loosely packaged patties after 1 week of refrigerated storage.

In this study, product in MAP had higher TBA values than AP ($P < 0.05$) perhaps due to high level of O₂ in MAP than in AP and the present of carbon dioxide in MAP favoured the dominance of a facultative anaerobic population spoilage bacteria and that enhances the oxidation process. Previous studies revealed a positive correlation between the microbial load and lipid oxidation in meat products (Sahoo and Anjaneyulu 1997, Kandeepan et al. 2009).

The higher TBA values of control sample compered to sample treated with rosemary extract were significantly different ($P < 0.05$) in both AP and MAP, while no significant differences were observed in VP at 30°C and 20°C and the results are positively correlated to storage time ($P < 0.05$). Similarly, Sebranek et al. (2005) reported that rosemary extracts offered considerable protection against lipid oxidation, and maintained the freshness of cooked pork sausages stored refrigerated.

Moreover, Fernández-López et al (2005) indicated that all rosemary extracts Rosemary oil extract (OR), rosemary water extract (WR) and rosemary oil and water extract (OWR) can be used to reduce lipid oxidation in a cooked beef meat product (meat ball), particularly WR which maintain the initial TBA values

during the 12 day storage period. In this work, the TBA for doner kebab treated with rosemary extract in all packaging conditions increased during storage, and that may be due to many reasons: type and concentration of rosemary used, cooking method, type of meat, fat content and finally storage temperature.

Many researchers have indicated the effectiveness of rosemary extracts in retarding lipid oxidation (Güntensperger et al. 1998, Sánchez-Escalante et al. 2001, Yu et al. 2002, Georgantelis et al. 2007). In addition, rosemary extracts have been reported to be more effective than the synthetic antioxidant compound (BHA/BHT) for delaying the oxidation process (Kenji and Mitsuo 1998, Sebranek et al. 2005).

According to Sebranek et al. (2005) rosemary extracts when applied to refrigerated cooked pork sausages at 2500 ppm concentration was more effective than the maximum permitted concentrations of BHA/BHT. This antioxidant property of rosemary is mainly due to rosmanol, rosmariquinone, rosmaridiphenol and carnosol (Kenji and Mitsuo 1998).

Comparisons between the storage temperature (30 and 20)°C, show that the oxidation rates (TBA values) were higher at 30°C than at 20°C on different treatments used. It was expected that higher temperatures would have enhanced the oxidation process. When the temperature is raised, the oxygen level becomes more critical in lipid oxidation in both MAP and air stored meats (Jakobsen and Bertelsen 2000).

5.9.6.2 Stability losses

The Rancimat method was used to determine the amount of oxidation (stability losses) of doner kebab slices treated with different treatment under (30 and 20) °C and results are illustrated in (Figures 5.10 - 5.12), and (Table 5.5). The quality loss index was calculated from the induction time of the sample (cooked doner kebab + sun flower oil) divided by the induction time of the control (sun flower oil).

As would be expected, the stability losses at 20°C are lower than those at 30°C. Similarly, Fennema (1996) and Bhattacharya et al. (1988), both reported that the rate of oxidation is positively correlated with storage temperature, (the rate of oxidation increased as the temperature increased). The effect of temperature on lipid oxidation was measured depending on the reaction rate at each temperature (Table 5.6) which was calculated by using zero order rate equation (eq. 5.6) (Man and Jones 1994).

$$ts = \frac{Q_o - Q_e}{K} \dots \dots \dots (5.6)$$

Where Q_o represented some initial value of the quality attribute, ts is the end of shelf life that is noticed by the quality attribute reaching a certain level Q_e , and K is the reaction rate. The equation can also be written as (eq.5.7).

$$ts = \frac{Q_i - Q_j}{K} \dots \dots \dots (5.7)$$

Where Q_i represents the quality loss at ts shelf life, Q_j is the initial quality loss at the beginning of the shelf life and k is the reaction rate. For instance, k at 20°C for aerobic packaging control sample APC was calculated with eq.5.8.

$Q_j = 1.402$ (at day 1 obtained from $y = 0.19x + 1.14$ (Figure 5.11)).

Qi= 1.607 (at day six obtained from $y = 0.19x + 1.14$ (Figure 5.11)).

$$k_{20^{\circ}\text{C}} = \frac{1.607 - 1.402}{6} = 0.034 \dots \dots \dots \text{(eq. 5.8)}$$

While at 30°C k value was 0. 082

Qj= 1.865 (at day one obtained from $y = 0.43x + 1.11$ (Figure 5.11)).

Qi= 2.479 (at day six obtained from $y = 0.43x + 1.11$ (Figure 5.11)).

Therefore, the ratio between $K_{20^{\circ}\text{C}}: K_{30^{\circ}\text{C}} = 1:2.4$ which means that the oxidation rate of APC cooked doner kebab was 2.4 times higher at 30°C than at 20°C.

Quality loss index of VPC and VPS at the two temperatures used during the storage time indicated that the increase in oxidations is significant ($P < 0.05$), while the difference in TBA value was not. This could be due to the different reactions of each method used (Hui 2006). In Rancimat method (accelerated oxidation test), during the measurement of the doner kebab sample a stream of air is passed through the fat sample contained in a sealed and heated reaction vessel. This treatment results in oxidation of the fat molecules, with peroxides initially being formed as the primary oxidation products. After some time the fatty acids are completely destroyed; the secondary oxidation products formed include low-molecular organic acids in addition to other volatile organic compounds. These are transported in the stream of air to a second vessel containing distilled water. The conductivity in this vessel is recorded continuously. The organic acids can be detected by the increase in conductivity. The time that elapses until these secondary reaction products appear is known as the induction time or Oil Stability Index (OSI). This method is based on the principle that aging and rancidification of a fat which is greatly accelerated by

aeration in a tube held at a constant elevated temperature (Hui 2006 , Casimir and Min 2008).

However, TBA method is frequently used for measuring the secondary oxidation products of lipid. MA (malonaldehyde) is a relatively minor product of oxidation of polyunsaturated fatty acid that reacts with TBA reagent to produce a pink complex with an absorption of 538 nm (Casimir and Min 2008). Therefore, some differences in the oxidation rate may be occurring.

5.10 Conclusions

This study provide a comprehensive picture of the composition and the shelf life of vacuum pack ready to eat doner kebab stored at 4°C. Vacuum packaging maintain the product stable for 11 weeks; sensory scores were all in the well-accepted region for the panel evaluation, both TVC and LAB were not achieved the unsatisfactory levels and TBA values was below the threshold limit.

Study an accelerate shelf life protocol, indicated that doner kebab shelf life is a function of temperate, storage conditions and spice addition.

Temperature is a critical factor affecting the oxidation rate of cooked doner kebab; the oxidation rate was 2.4 times higher at 30°C than at 20°C.

The use of rosemary extracts and vacuum packaging in high fat cooked meat product (kebab) effectively decreased the oxidation rate, thereby extending the shelf life of the product. Rosemary oleoresin and vacuum pack could be used in the food industry to improve the shelf life of high fat meat products.

Chapter 6

6 Antioxidant and Antibacterial Activity of Spice Extracts Applied to VP and MAP Lamb Doner Kebab

6.1 Introduction

Doner Kebab is one of the most appreciated traditional Medill East meat products, needs to undergo an inevitable technological evolution to satisfy regulatory safety requirements and the customer expectations.

Efforts to find acceptable ways to maintain good quality and safety RTE meat products are of great importance. Accordingly, packaging technologies have been developed by food processors and researchers to reach this point (Leistner and Gould 2002).

Food packaging has been limited to preservation and protection of food from environmental factors including chemical, physical and biological influences up to the point of consumption. This emphasizes retarding spoilage, extending shelf-life, and preserving the quality of packaged RTE food (Brody et al. 2008). Vacuum packed RTE cooked meat products have a good safety record (Babji et al. 2000, Soldatou et al. 2009), whereas the microbial safety of modified atmosphere pack (MAP) are mainly affected by both gas atmosphere and storage temperature (Phillips 1996, Skandamis and Nychas 2002). using this technology does not eliminate undesirable psyrchotrophic pathogenic bacteria from these products, thus the concern about the possible growth remains a limiting factor to extra expansion of this method (Murcia et al. 2003).

Therefore, the addition of extracts from common culinary herbs, spices and aromatic plants is the key factor for doner kebab development of this RTE cooked meat products.

In addition, according to several studies (Lai and Roy 2004, Majhenic et al. 2007) the antimicrobial and the antioxidant activity of the spice extracts can contribute to the shelf-life extension of such perishable products.

Therefore, the main objectives of this work were to determine the combined effect of culinary spice extracts and packaging conditions on the shelf life of RTE doner kebab. The evaluation of the doner kebab shelf life was based on the microbiological counts of total viable counts and lactic acid bacteria, pH, a_w , colour measurement, and TBA, sensory evaluation as well as the presence of pathogenic bacteria.

6.2 Materials and Methods

6.2.1 Ethical approval

The Human Ethics committee of the Faculty of Science /University of Plymouth approved the sensory evaluation protocol as detailed in Section 3.9.

6.2.2 Natural extract

Based on the results obtained from Chapter 4, rosemary oleoresin, cinnamon oleoresin and sumac alcoholic extracts were chosen for application into lamb doner kebab; rosemary and cinnamon oleoresin were provided by Kalsec as in Section 3.1 and sumac alcoholic extracts were obtained by the method described by Fazeli et al.(2007) as in Section 3.1.1.2.

6.2.3 Product preparation

Doner kebab rolls of 15 kg as detailed in Section 3.10.1 were thawed over night at $4\pm1^\circ\text{C}$ (Williams Refrigeration and Air-conditioning, installation by general

refrigeration, Limited). After thawing, the rolls were divided into 2 batches: one was used for sensory evaluation test and the other to study the shelf life of the product. For sensory evaluation (ranking test), the batch was divided into 12 blocks, each of about 600g, and randomly assigned to 11 treatments: Rosemary oleoresin, sumac alcoholic extract and cinnamon oleoresin at three concentrations (0.05, 0.125 and 0.2 ml/100 g) and a combination of (1:1:1) mixture of rosemary, cinnamon and sumac (high concentration level); while two blocks were left without spices as a control. Batches were homogenised separately in a bowl mixer with a spiral dough hook (Silverson, Birmingham, UK) at medium speed (80 rpm) for 5 min. For each treatment, the corresponding extracts were added at the concentrations mentioned above, and then mixed again for 5 min. Kebab batches were formed and cooked as detailed in Section 3.11. Then the slices were cooled down to reach 22°C, maintained warm 65.5°C in an oven between 10-20 min, then served to the consumer panel to evaluate the products by measuring the acceptability of colour, flavour, test, overall appearance, overall acceptance and spiciness. .

The other batch (10 kg), was divided into 8 blocks, each one about 1250 g which was treated with the corresponding spice extract of two level (0.05 and 0.2 ml/100 g) and a combination of (1:1:1) mixture of rosemary, cinnamon and sumac (high concentration level); while one block was left without spices as a control. Followed by the mixing, formulation and cooking processes as mentioned in Section 3.11. After cooking, the kebab slices were cooled down immediately in ice water bath to reach a product temperature below 12°C.

6.2.4 Storage conditions

After the kebab samples reached the packing temperature they were divided equally into two groups: one group was vacuum packed and the other group was modified atmosphere packed as detailed in Section 3.12.

The slices were stored in a cooler at $4\pm1^{\circ}\text{C}$ (Williams Refrigeration and Air-conditioning, installation by general refrigeration, Limited) for 12 weeks. Sampling and storage conditions records from each treatment were taken at 0, 2, 4, 6, 8, 10 and 12 weeks and every sample was analysed as follows.

6.2.5 Doner kebab analyses

6.2.5.1 pH value

The pH value was evaluated by using a microprocessor pH meter as in Section 3.13.2.3.

6.2.5.2 Water activity

Water activity of doner kebab slice was determined as in Section 3.13.2.4.

6.2.5.3 TBA values

The secondary lipid oxidation products from cooked VP and MAP doner kebab stored at 4 °C was assessed as in Section 3.13.2.2.

6.2.5.4 Microbiological analysis

Doner kebab slices aseptically analysis as detailed in Section 3.13.1.

Additionally, growth data obtained from TVC and LAB for both VP and MAP were fitted to a primary model, Baranyi function, Baranyi and Roberts (1994) by using DMFit software (Institute of Food Research, Norwich, UK) to evaluate the kinetic growth parameter under different conditions used.

Baranyi function:

$$N_t = N_0 + \mu_{\max} \times A_t + \ln \left(\frac{1 + \exp(\mu_{\max} \times A_t) - 1}{1 / \exp(N_{\max} - N_0)} \right) \dots \dots \dots (6.1)$$

Where N_t , the bacterial population at any time t (ln CFUg⁻¹); N_{\max} and N_0 , the maximum and initial population level, respectively (ln CFUg⁻¹); μ_{\max} , the maximum specific growth rate (d⁻¹); and A_t , an adjustment function as defined by Baranyi and Roberts (1994), considered to account for the physiological state of the cells. The statistical index of the coefficient of determination (R^2) was used in the evaluation of model performance (Mataragas et al. 2006).

6.2.5.5 Sensory analysis

Sensory-derived effects of adding each of the spice extracts to the doner kebab were evaluated following standard guidelines of International Standards ISO 6658-1995 and ISO 6564-1985 (British Standards 5929 1986a, b).

Two methods were used to evaluate the products:

6.2.5.5.1 Ranking test (preference test)

This method was used to compare several samples according to preference evaluating of a single attribute individually, such as spiciness, acceptances, and freshness. The ranking test was considered to be the simplest method to perform such comparison (Morten Meilgaard et al. 1999).

Control and spice extracts samples were assessed by consumer panels, which were recruited from the staff and students of Plymouth University. This evaluation took place only once. Samples were prepared by cooking as described earlier and maintained warm in an oven until testing within 3–8 min. All sensory work was carried out in the sensory laboratory/ food and nutrition suit at Plymouth University.

During the evaluation test, the panellists were placed in private booths under fluorescent light, then doner kebab slices were served at room temperature, each panellist evaluated the control and treatment samples; the samples were coded, and placed separately on a small white plate and presented in random order for each panellist. Water at room temperature was provided for cleansing the palate between samplings for each panellist. The panellists were asked to evaluate the acceptability by ordering the samples for each of the following attributes: colour, taste, aroma, spiciness, overall appearance and overall acceptance of the samples from the ballots (Appendix B3), the rank sums were calculated and the absolute difference between the rank sums were determined according to Friedman's analysis. Friedman's values were computed as follow:

$$F = \frac{12}{JP(P+1)} (R_1^2 + R_2^2 + \dots R_p^2) - 3J/(P+1) \dots \dots \dots (6.2)$$

Where J is the number of assessor, P is the number of product, R_1 - R_p : are the rank sum attributed to the P sample for the J assessor. The computed F values were compared with a critical value from the F test table at level of 0.05 and 0.01.

6.2.5.5.2 Scoring test (Hedonic scale)

Doner kebab samples were sensory evaluated as in Section 3.13.3.1.

Panellists were asked to heat the samples in a microwave oven for 1 minute before evaluation of the sample (Appendix B2).

6.2.5.6 Statistical analysis

The results were statistically evaluate as in Section 3.14. Additionally, linear and polynomial regression was computed for TBARS data to evaluate the trend of lipid oxidation as a function of storage time progress.

For sensory evaluation (ranking test), results were de-codified and rank sums computed, followed by Friedman test for treatment comparisons.

For microbiological data, results were transformed to logarithms before analyses, and data were reported as means, which were fitted to the primary model, Baranyi function (Baranyi and Roberts 1994) by using DMFit manual Version 2.0 (Institute of Food Research, Norwich, UK).

6.3 Results

6.3.1 pH value

Changes in pH values of doner kebab samples stored under VP and MAP at 4 °C are shown in (Figures 6.1 and 6.2). The results indicated that pH values change significantly over time and affected by spice extracts addition and the packaging condition ($P < 0.05$). For the VP samples, both spice extracts and storage time significantly decreased the pH values. SH had a pH below 5.8 at the end of the experiment and this was significantly lower ($P < 0.05$) than the pH of other treatments Figure 6. 1.

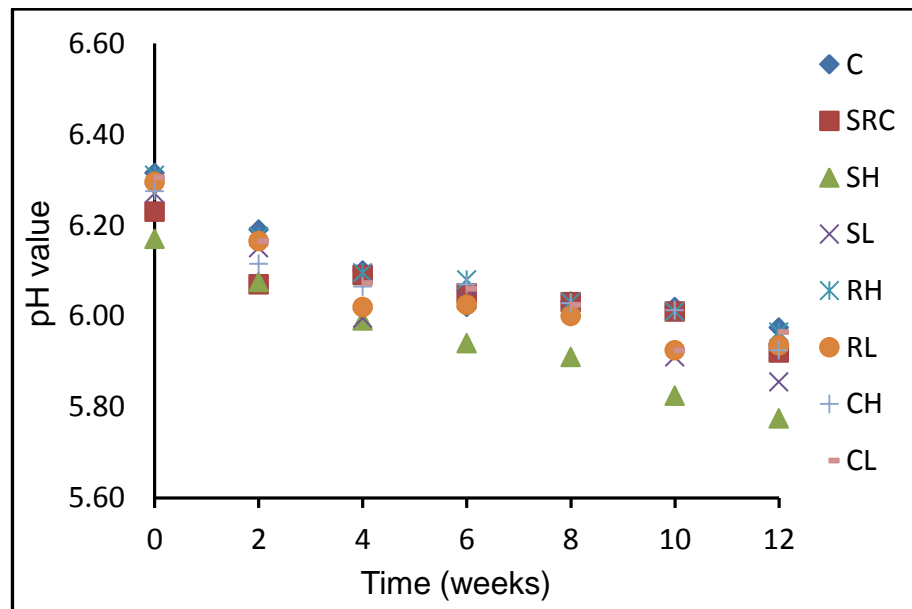


Figure 6.1: pH values in the refrigerated vacuum pack ready to eat doner kebab with added spice extracts over storage time. C: control (without spice), SRC: combination of sumac, rosemary and cinnamon, SH: sumac at high level, SL: sumac at low level, RH: rosemary at high level, RL: rosemary at low level, CH: cinnamon at high level, CL: cinnamon at low level. Data are given as a mean of three reading.

For the MAP a different pattern was observed, all samples increased in pH as storage time progress, and the significant increase started from week 6 to reach 6.3 for SRC, CH and RL, and 6.27, 6.26, 6.22, 6.19, 6.17 for CL, RH, Control, SL and SH respectively at the end of the experiment ($P < 0.05$) Figure 6. 2.

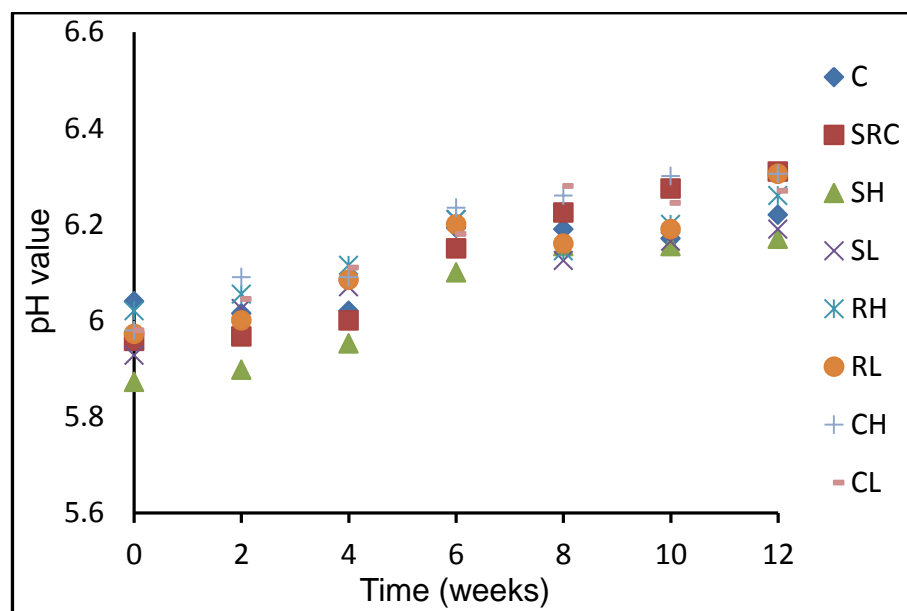


Figure 6.2: pH values in refrigerated modified atmosphere pack ready to eat doner kebab with added spice extracts over storage time. For sample key, see Figure 6. 1.

6.3.2 Water activity

The changes in the water activity are illustrated in Figure 6.3 and 6.4 respectively.

In general, the water activity in samples decreased ($P < 0.05$) during storage for both packaging methods used. From week 0 -12 all spice extracts treated samples had lower a_w values than controls ($P < 0.05$) with the lowest values obtained from rosemary extracts (RH, RL).

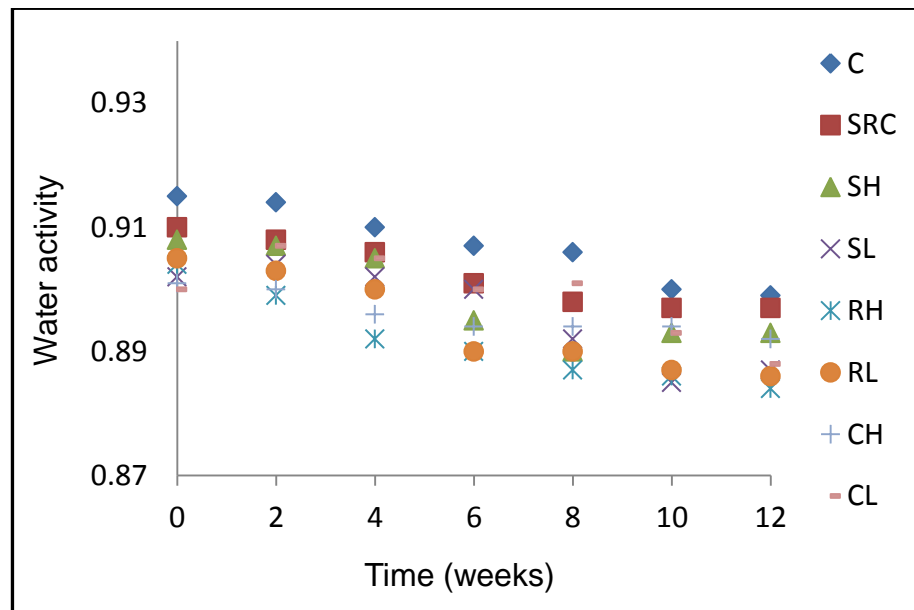


Figure 6.3: Water activity in refrigerated vacuum pack ready to eat doner kebab with added Spice extracts, during storage time. For sample key, see Figure 6.1

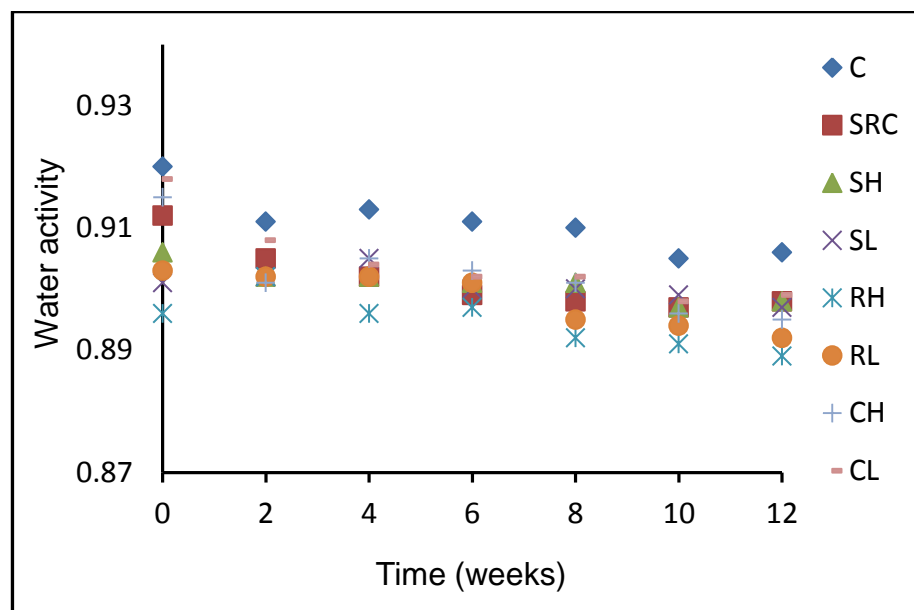


Figure 6.4: Water activity in refrigerated vacuum pack ready to eat doner kebab with added Spice extracts, during storage time. For sample key, see Figure 6.1

6.3.3 Microbiological analysis

The effects of spice extracts, packaging conditions and time on the growth of TVC and LAB are shown in Figures 6.5–6.8.

6.3.3.1 Total viable count (TVC)

At week 0, the CH, CL, RH, RL and SRC samples showed lower aerobic viable bacterial count (TVC) than control samples ($P < 0.05$) in VP doner kebab, then the bacterial population increased steadily during storage time, with significant differences between all spice extracts samples and control ($P < 0.05$) except SH and SL (Figure 6.5.).

Lowest TVC were obtained from CH and SRC samples ($P < 0.05$), both reduced the final bacterial count for about 2 log cycle compared with control, SH, and SL and 1 log cycle as opposed with RH, RL, and CL.

For MAP samples, lower initial aerobic viable count was obtained from spice extracts samples compared with control samples ($P < 0.05$) with no significant difference between spice treatments ($P > 0.05$). Then during the storage TVC showed the same scenario observed under VP for all samples.

6.3.3.2 Lactic acid bacterial count (LAB)

For LAB both VP and MAP showed a decline trend during storage time for spice extracts samples, whereas, for control samples LAB counts were slightly increased (Figures 6.7 and 6.8).

The antibacterial activity of spice extracts application in VP doner kebab samples started from week 0 and continue to reach the no detection level at different time. CH had a higher effect ($P < 0.05$), reduced the initial LAB counts by 1.6 log cycle compared with control and reached the not detection level at week 2.

For MAP samples, the initial counts of LAB were started from $3 \pm 0.3 \log \text{CFUg}^{-1}$ for all spice treated samples then gradually decreased to reach the no detection level at week 12 for all samples except SRC and CH, which reached the no detection level at week 6.

No growth of Enterobacteriaceae, *E. coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, or moulds and yeasts was detected in both VP and MAP.

6.3.3.3 DMFit model (Baranyi and Roberts model)

The growth kinetic parameters of TVC and LAB and their coefficients of determination in doner kebab samples stored at 4°C at different packaging conditions are shown in (Figures 6.5– 6.8) and (Tables 6.1 and 6.2).

In general, all growth data were satisfactorily fitted to growth curves obtained from the Baranyi and Roberts' model for all the treatments used with high R^2 values and low standard errors. The growth kinetic parameter for TVC revealed that no significant differences between VP and MAP samples. Optimum growth rate was shown in control samples in both VP and MAP condition; whereas there was a lower growth rate obtained from cinnamon (CH) and SRC. Both significantly reduced the growth rate of TVC comparing with other treatments in VP and MAP, and also they reduced the maximum final growth by 2 log cycles (Table 5.1). The growth kinetic estimate indicated that TVC attend higher values for both initial cell density (y_0) and final cell density (y_{End}) than LAB regardless to storage time, with the higher values for y_{End} observed in the control sample.

For LAB, all spice extracts decreased LAB counts over time ($P > 0.05$), with negative growth rate values between 0.11 to 0.78 for VP, but just down to 0.3 for MAP. Whereas, for the control samples LAB counts increased regarding to the storage time ($P < 0.05$), a with growth rate of 0.05 and 1.16 for VP and MAP

respectively. The high level of cinnamon extract addition (CH) controlled LAB levels below detection limit from week 2 and highly reduced the bacterial count from the time of addition in VP samples, whereas in MAP cinnamon reduced the level of LAB from week 0 and reached the not detection level at week 6.

➤ TVC-VP

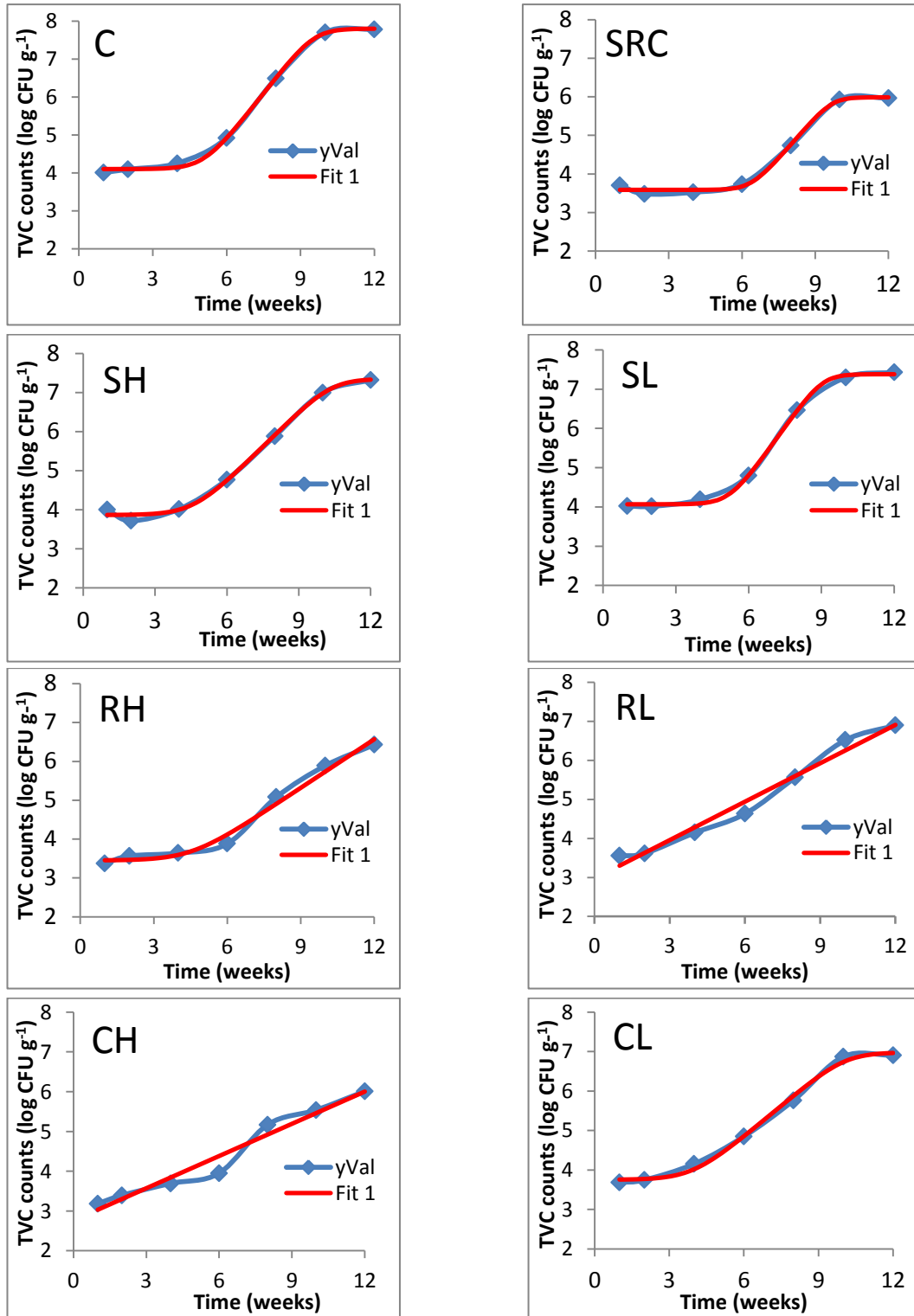


Figure 6.5: Total viable bacteria (TVC) counts (log CFUg⁻¹) in vacuum pack (VP) doner kebab slices with added spice extracts, during storage time (weeks). C: Control, SRC: Combination of (sumac, rosemary and cinnamon), SH: Sumac high concentration, SL: Sumac low concentration, RH: Rosemary high concentration, RL: Rosemary low concentration, CH: Cinnamon high concentration, CL: Cinnamon low concentration.

➤ TVC.- MAP

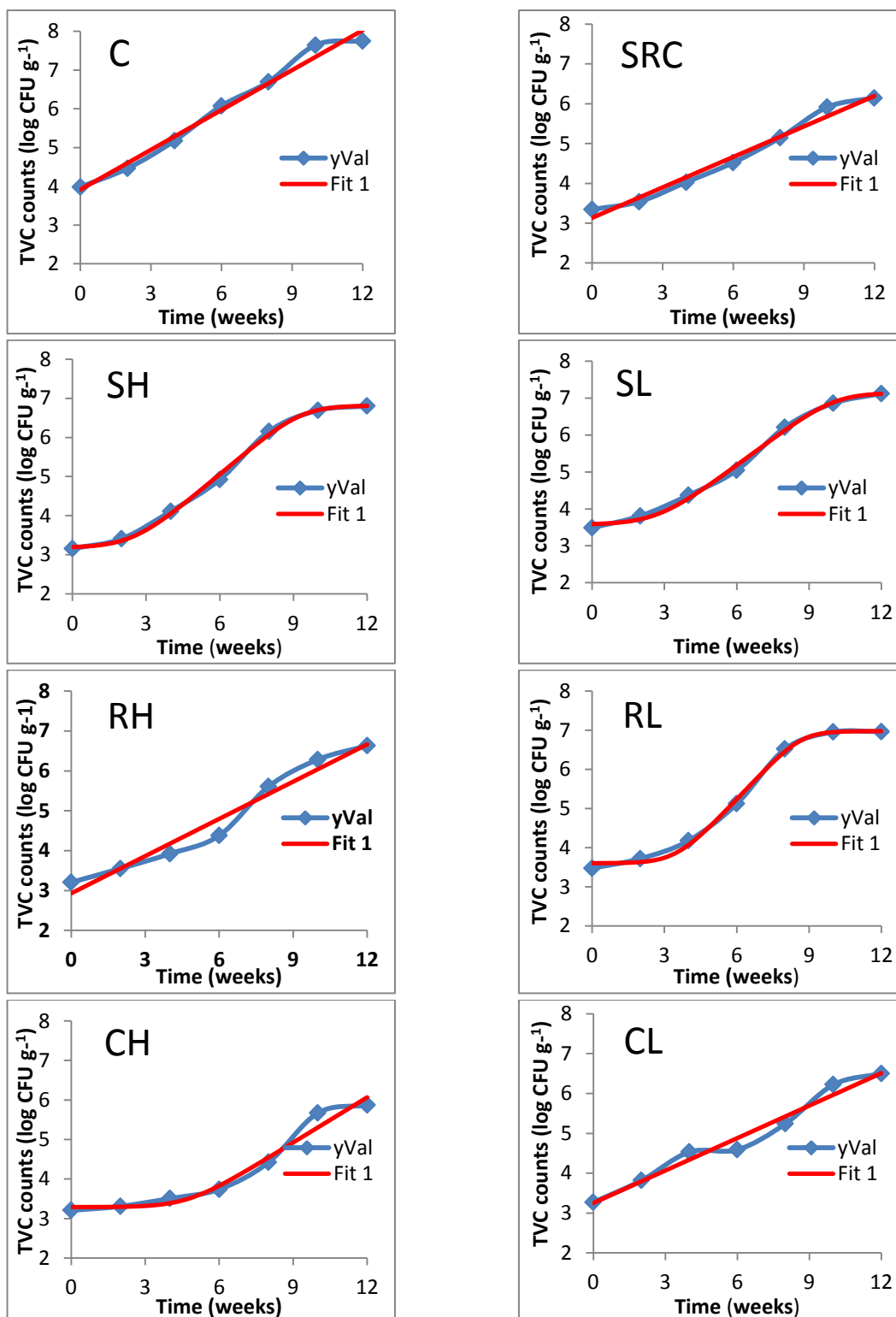


Figure 6.6: Total viable bacteria (TVC) counts (log CFUg⁻¹) in modified atmosphere pack doner kebab slices with added spice extracts, during storage time (weeks). For sample key see Figure 6.5

➤ LAB - VP

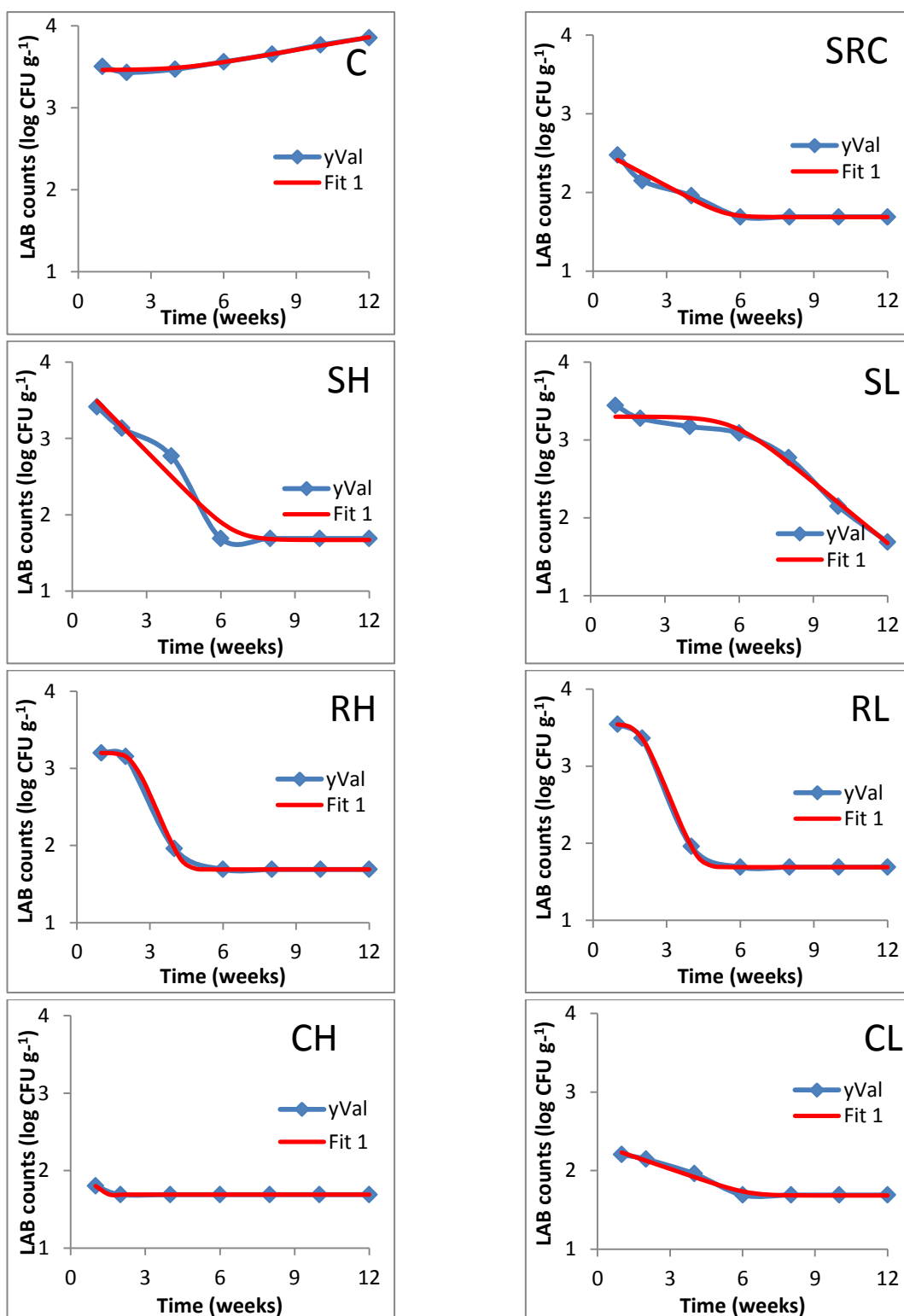


Figure 6.7: Lactic acid bacteria (LAB) counts (log CFUg⁻¹) in vacuum pack (VP) doner kebab slices with added spice extracts, during storage time (weeks). For sample key, see Figure 6.5.

➤ LAB - MAP

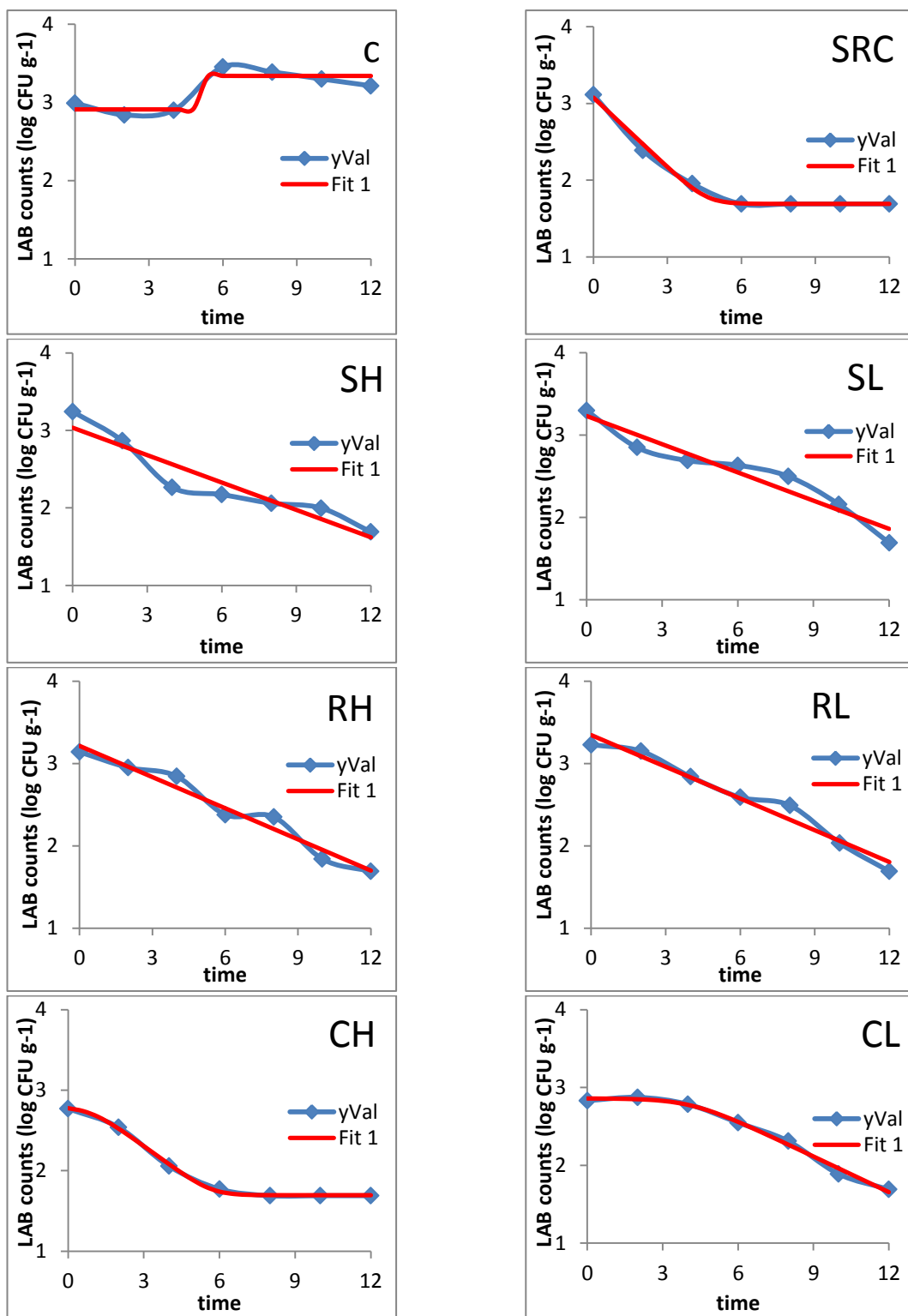


Figure 6.8: Total viable bacteria (TVB) counts (log CFUg⁻¹) in modified atmosphere pack doner kebab slices with added spice extracts, during storage time (weeks). For sample key, see Figure 6.6.

Table 6.2: The effect of packaging conditions and spice extracts treatments on TVC in ready to eat doner kebab stored at 4°C for three months.

| Packaging Type | Treatments | Modelling parameters | | | | | | | | |
|----------------|------------|----------------------|--------|-------|------|------|-------|---------|----------------|------|
| | | mCurv* | nCurv* | rate* | lag* | y0* | yEnd* | se(fit) | R ² | inoc |
| VP | C | 10 | 1 | 0.83 | 4.0 | 4.10 | 7.80 | 0.08 | 0.990 | 4.01 |
| | SRC | 10 | 1 | 0.27 | 5.3 | 3.59 | 5.99 | 0.11 | 0.990 | 3.70 |
| | SH | 10 | 1 | 0.60 | 3.6 | 3.87 | 7.35 | 0.12 | 0.990 | 4.00 |
| | SL | 10 | 1 | 0.86 | 4.2 | 4.07 | 7.38 | 0.08 | 0.990 | 4.03 |
| | RH | 0. | 1 | 0.33 | 4.0 | 3.30 | 6.57 | 0.19 | 0.980 | 3.37 |
| | RL | 0 | 0 | 0.45 | | 3.46 | 6.90 | 0.22 | 0.970 | 3.55 |
| | CH | 0 | 0 | 0.26 | | 3.13 | 6.08 | 0.25 | 0.940 | 3.18 |
| | CL | 10 | 1 | 0.33 | 2.94 | 3.46 | 6.98 | 0.14 | 0.950 | 3.69 |
| MAP | C | 0 | 0 | 0.74 | | 3.91 | 7.75 | 0.21 | 0.980 | 3.98 |
| | SRC | 0 | 0 | 0.26 | | 3.13 | 6.15 | 0.17 | 0.976 | 3.34 |
| | SH | 10 | 1 | 0.53 | 2.0 | 3.20 | 6.81 | 0.11 | 0.990 | 3.15 |
| | SL | 10 | 1 | 0.59 | 2.0 | 3.59 | 7.14 | 0.13 | 0.992 | 3.49 |
| | RH | 0 | 0 | 0.31 | | 2.93 | 6.63 | 0.29 | 0.956 | 3.20 |
| | RL | 10 | 1 | 0.55 | 3.0 | 3.60 | 6.98 | 0.13 | 0.992 | 3.47 |
| | CH | 0 | 1 | 0.27 | 4.0 | 3.25 | 5.97 | 0.24 | 0.954 | 3.21 |
| | CL | 0 | 0 | 0.39 | | 3.34 | 6.50 | 0.21 | 0.969 | 3.27 |

*Primary parameter: (rate: potential maximum growth rate, lag: lag time, y0: initial point of the sigmoid curve, yEnd: upper or lower asymptote of the sigmoid curve depending on growth trend), *Curvature parameters: (nCurv: at the beginning of the linear phase, mCurv: at the end of the linear phase), se (fit): Standard error of fitting, R²: Adjusted R-square statistics of the fitting, inoc: initial bacterial level

Table 6.3: The effect of packaging conditions and spice extracts treatments on LAB in ready to eat doner kebab stored at 4°C for three months

| Packaging Types | Treatments | Modelling parameters | | | | | | | | |
|-----------------|------------|----------------------|-------|-------|------|------|------|----------|----------------|------|
| | | mCurv | nCurv | rate | lag | y0 | yEnd | se (fit) | R ² | inoc |
| VP | C | 0 | 1 | 0.05 | 3.27 | 3.46 | | 0.03 | 0.97 | 3.50 |
| | SRC | 10 | 0. | -0.17 | | 2.41 | 1.69 | 0.06 | 0.96 | 2.48 |
| | SH | 10 | 0 | -0.33 | | 3.49 | 1.67 | 0.18 | 0.95 | 3.41 |
| | SL | 0 | 1 | -0.26 | 4.78 | 3.30 | | 0.10 | 0.98 | 3.44 |
| | RH | 10 | 1 | -0.73 | 1.26 | 3.20 | 1.69 | 0.00 | 1.00 | 3.20 |
| | RL | 10 | 1 | -0.78 | 0.91 | 3.54 | 1.69 | 0.00 | 1.00 | 3.54 |
| | CH | 10 | 0 | -0.24 | | 1.80 | 1.69 | 0.00 | 1.00 | 1.80 |
| | CL | 10 | 0 | -0.11 | | 2.23 | 1.68 | 0.04 | 0.98 | 2.20 |
| MAP | C | 10 | 1 | 1.16 | 4.94 | 2.91 | 3.34 | 0.12 | 0.75 | 2.99 |
| | SRC | 10 | 0 | -0.30 | | 3.08 | 1.69 | 0.05 | 0.99 | 3.12 |
| | SH | 0 | 0 | -0.12 | | 3.04 | | 0.19 | 0.87 | 3.24 |
| | SL | 0 | 0 | -0.11 | | 3.23 | | 0.15 | 0.92 | 3.30 |
| | RH | 0 | 0 | -0.13 | | 3.21 | | 0.11 | 0.96 | 3.14 |
| | RL | 0 | 0 | -0.13 | | 3.35 | | 0.11 | 0.96 | 3.23 |
| | CH | 10 | 1 | -0.23 | 0.94 | 2.78 | 1.70 | 0.03 | 1.00 | 2.77 |
| | CL | 0 | 1 | -0.15 | 4.12 | 2.86 | | 0.05 | 0.99 | 2.83 |

*Primary parameter: (rate: potential maximum growth rate, lag: lag time, y0: initial point of the sigmoid curve, yEnd: upper or lower asymptote of the sigmoid curve depending on growth trend, *Curvature parameters: (nCurv: at the beginning of the linear phase, mCurv: at the end of the linear phase), se(fit): Standard error of fitting, R²: Adjusted R-square statistics of the fitting, inoc: initial bacterial level.

6.3.4 TBA value

The effect of spice extracts on secondary lipid oxidation is illustrated in (Figures 6.9 and 6.10). TBA values of VP sample, for all treatments, were lower than MAP sample ($P < 0.05$).

In MAP storage, at week 0, all spice extracts treated samples had lower TBA values than control samples ($P < 0.05$). Whereas, in VP lower TBA value was obtained from RH, CH, SRC, RL and CL compared with control samples ($P > 0.05$). At week 2, 4, 6, 8, 10 and 12 for both VP and MAP higher TBA value was achieved by SH and SL ($P < 0.05$) while, lowest value obtained from RH, CH, and SRC with significant differences between both packaging method ($P < 0.05$).

The oxidation rate was determined by using a polynomial regression (linear and quadratic) for both packaging methods in RTE doner kebab, both models were well fitted to the TBA data with R^2 more than 0.93 in VP and higher than 0.83 in MAP (Table 6.5 and 6.6). It is evident that the oxidation rate in doner kebab sample stored under VP condition was lower than MAP.

Table 6.7 showed the difference between the two models used and the measuring error of TBA values at the end of the experiment. The differences were smaller than the measuring error in VP samples, whereas in MPA samples were greater.

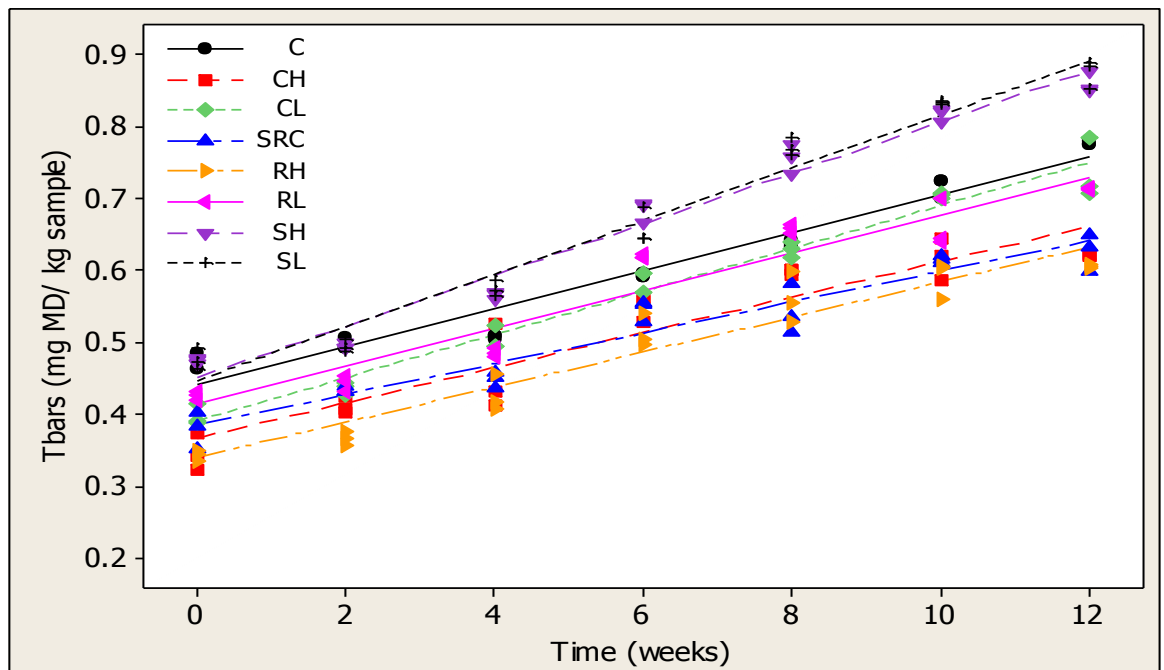


Figure 6.9: Rancidity (TBA) evolution in VP doner kebab with different natural extracts at high and low levels added, during storage time. For sample key, see Figure 6.1

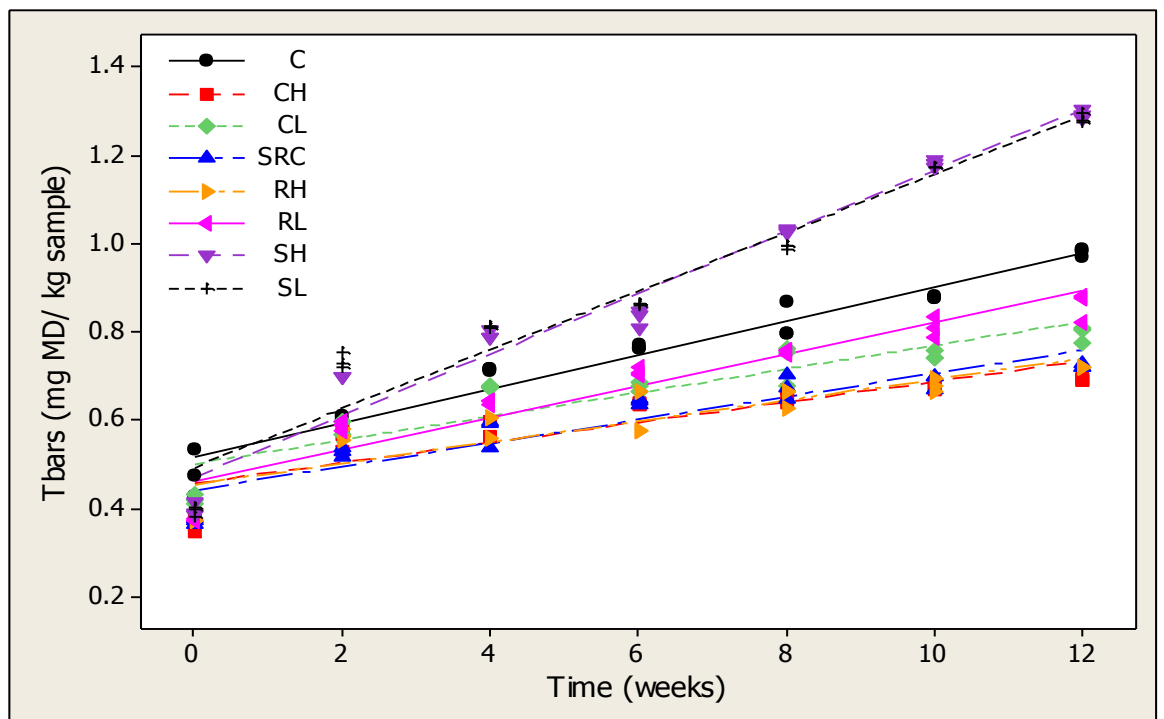


Figure 6.10: Rancidity (TBA) evolution in MAP doner kebab with different natural extracts at high and low levels added, during storage time. For sample key, see Figure 6.1.

Table 6.4: Rate of oxidation deterioration* for vacuum pack ready to eat doner kebab stored at 4°C during 12 weeks.

| Treatments | Order 1 | | | Order 2 | | | |
|------------|---------|-------|----------------|---------|-------|--------|----------------|
| | b[0] | b[1] | r ² | b[0] | b[1] | b[2] | r ² |
| C | 0.453 | 0.029 | 0.978 | 0.449 | 0.010 | 0.001 | 0.987 |
| CH | 0.361 | 0.025 | 0.935 | 0.335 | 0.044 | -0.002 | 0.981 |
| CL | 0.391 | 0.030 | 0.991 | 0.386 | 0.033 | 0.000 | 0.991 |
| SRC | 0.384 | 0.024 | 0.957 | 0.376 | 0.026 | 0.000 | 0.961 |
| RH | 0.340 | 0.024 | 0.960 | 0.324 | 0.034 | -0.001 | 0.972 |
| RL | 0.415 | 0.026 | 0.932 | 0.403 | 0.034 | -0.001 | 0.938 |
| SH | 0.451 | 0.035 | 0.979 | 0.451 | 0.035 | 0.000 | 0.979 |
| SL | 0.447 | 0.036 | 0.979 | 0.452 | 0.034 | 0.000 | 0.979 |

*Oxidation deterioration obtained by polynomial linear (order 1) and polynomial quadratic (order 2) regression, b[0]= the intercept, b[1] and b[2] = regression (oxidation rate), r²= the regression coefficient (the coefficient of determination). For sample key, see Figure 6. 1.

Table 6.5: Rate of oxidation deterioration* for modified atmosphere pack ready to eat doner kebab stored at 4 °C during 12 weeks.

| Treatments | Order 1 | | | Order 2 | | | |
|------------|---------|-------|----------------|---------|-------|--------|----------------|
| | b[0] | b[1] | r ² | b[0] | b[1] | b[2] | r ² |
| C | 0.489 | 0.042 | 0.949 | 0.450 | 0.065 | -0.002 | 0.974 |
| CH | 0.459 | 0.022 | 0.848 | 0.393 | 0.063 | -0.004 | 0.941 |
| CL | 0.533 | 0.024 | 0.893 | 0.493 | 0.048 | -0.002 | 0.972 |
| SRC | 0.442 | 0.026 | 0.882 | 0.39 | 0.056 | -0.003 | 0.977 |
| RH | 0.455 | 0.024 | 0.830 | 0.414 | 0.048 | -0.002 | 0.900 |
| RL | 0.466 | 0.036 | 0.896 | 0.407 | 0.071 | -0.003 | 0.966 |
| SH | 0.463 | 0.069 | 0.951 | 0.456 | 0.074 | -0.000 | 0.913 |
| SL | 0.493 | 0.066 | 0.949 | 0.465 | 0.083 | -0.001 | 0.954 |

*Oxidation deterioration obtained by polynomial linear (order 1) and polynomial quadratic (order 2) regression, b[0]= the intercept, b[1] and b[2] = regression (oxidation rate) r²= the regression coefficient (the coefficient of determination), For sample key, see Figure 6.1.

Table 6.6: Comparison of polynomial regression parameter R^2 ; linear and polynomial quadratic models at time 12.

| Storage type | Treatment | R^2 value | | | |
|--------------|-----------|-------------|--------|------------|-------|
| | | Order1 | Order2 | Difference | Stdev |
| VP | C | 0.958 | 0.987 | 0.030 | 0.003 |
| | CH | 0.935 | 0.981 | 0.046 | 0.001 |
| | CL | 0.990 | 0.991 | 0.001 | 0.041 |
| | SRC | 0.957 | 0.961 | 0.004 | 0.026 |
| | RH | 0.960 | 0.972 | 0.012 | 0.002 |
| | RL | 0.932 | 0.938 | 0.006 | 0.002 |
| | SH | 0.979 | 0.979 | 0.000 | 0.014 |
| | SL | 0.979 | 0.979 | 0.001 | 0.019 |
| MAP | C | 0.949 | 0.974 | 0.025 | 0.008 |
| | CH | 0.848 | 0.941 | 0.093 | 0.019 |
| | CL | 0.893 | 0.972 | 0.079 | 0.018 |
| | SRC | 0.882 | 0.977 | 0.095 | 0.003 |
| | RH | 0.830 | 0.900 | 0.070 | 0.001 |
| | RL | 0.896 | 0.966 | 0.070 | 0.035 |
| | SH | 0.950 | 0.913 | 0.037 | 0.011 |
| | SL | 0.949 | 0.954 | 0.005 | 0.010 |

For sample key, see Figure 6.1.

6.3.5 Sensory evaluation Shelf life trail

6.3.5.1 Ranking test (consumer panels)

The evaluation of the ranking test is summarized in (Figure 6.11). Differences between treatments were not perceived ($P > 0.05$) for the acceptability of colour, appearance, aroma and overall acceptance. Only taste acceptability and spiciness indicated significant differences ($P < 0.05$) between the different treatments.

The ranking test for the acceptability of the taste and the spiciness of the samples followed this ascending order with significant differences at 5% and 1% confidence interval (samples in the same line within the same colour line were not significantly different).

Taste acceptability ranking

CONTROL>SH>SRC>CL>SM>RL> RM >SL>CM>CH>RH 5% confidence



CONTROL>SH>SRC>CL>SM>RL> RM >SL>CM>CH>RH 1% confidence



Spiciness acceptability ranking

SRS>CH>CM>CL>SH>SM>CONTROL>RH>RM>SL>RL 5% confidence



SRS>CH>CM>CL>SH>SM>CONTROL>RH>RM>SL>RL 1% confidence



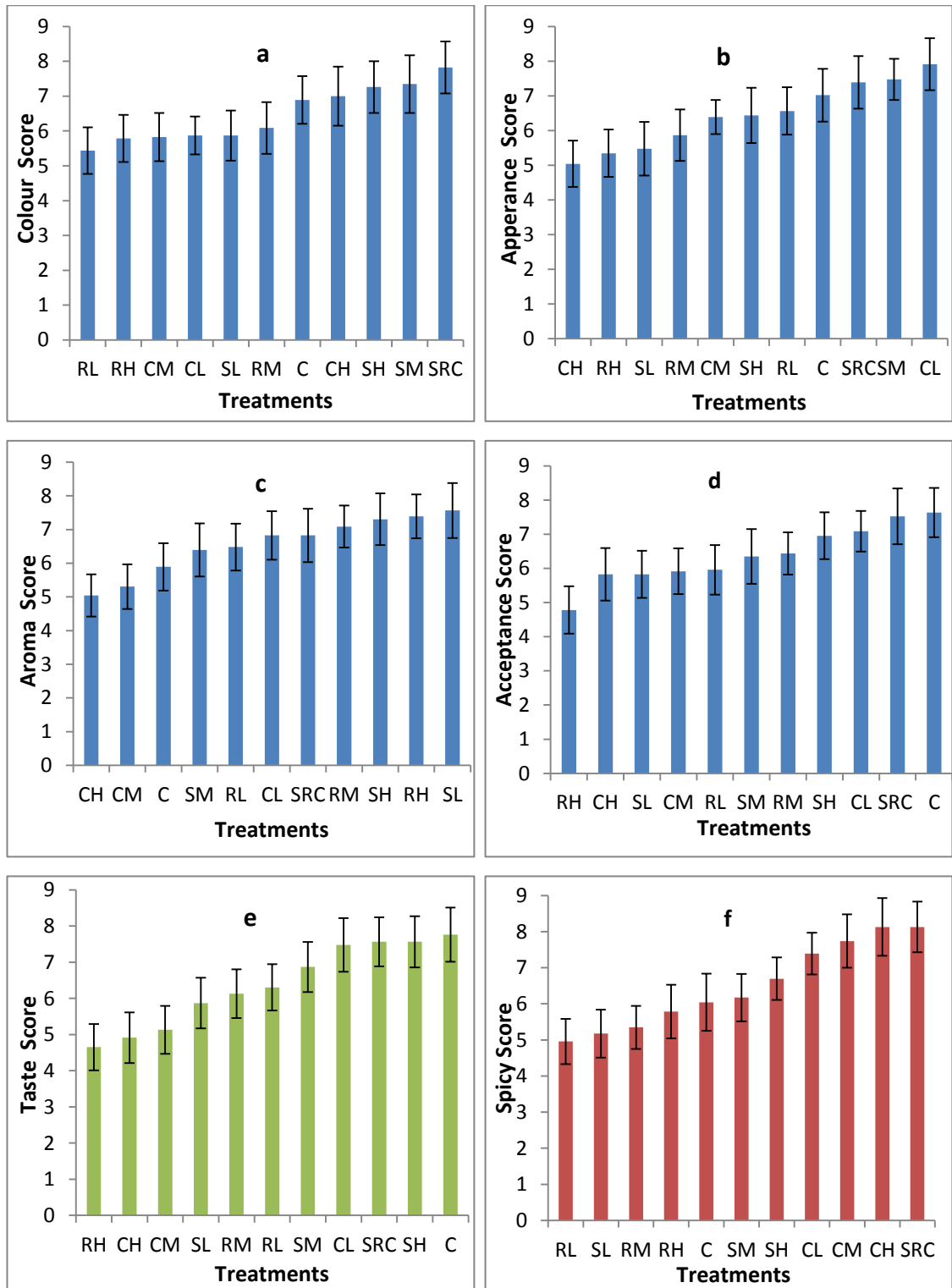


Figure 6.11: Sensory evolution (Ranking test) of ready to eat doner kebab for the acceptability of colour (a), overall appearance (b), aroma (c), overall acceptance (d), taste (e), and for spiciness (f). For sample key, see Table 6. 1

6.3.5.2 Hedonic test (semi trained panel)

Sensory evaluation results Figure 6.12 revealed that all attributes changed over time ($P < 0.05$). Packaging conditions (VP and MAP) had significant effects on colour, texture and overall appearance ($P < 0.05$); in general, VP showed higher scores for all attributes in comparison with MAP storage condition (Figure 6.12).

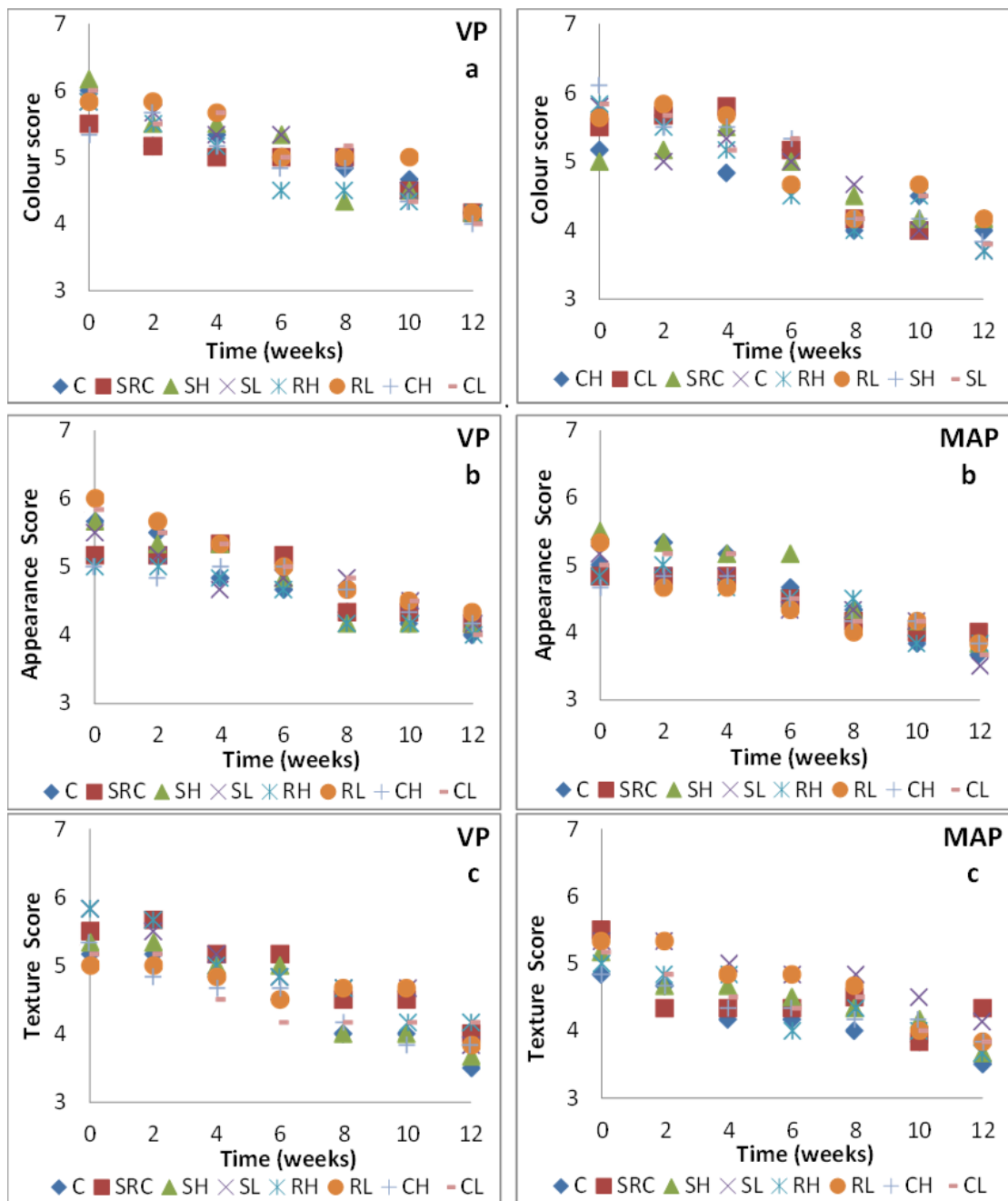


Figure 6.12: Evolution of sensory attribute (shelf life evaluation) of ready to eat doner kebab stored at 4 °C under vacuum pack (VP) and modified atmosphere pack (MAP) for colour (a), overall appearance (b), texture (c). The sensory questionnaires measured intensity on a 7-point balanced hedonic scale as follow: 7= very acceptable, 6= moderately acceptable, 5=slightly acceptable, 4= neither acceptable nor unacceptable, 3= slightly unacceptable, 2=moderately unacceptable and 1= very unacceptable.

Regarding spice extracts treatment, significant effects were observed in aroma and overall acceptance scores only ($P < 0.05$) (Figure 6.13).

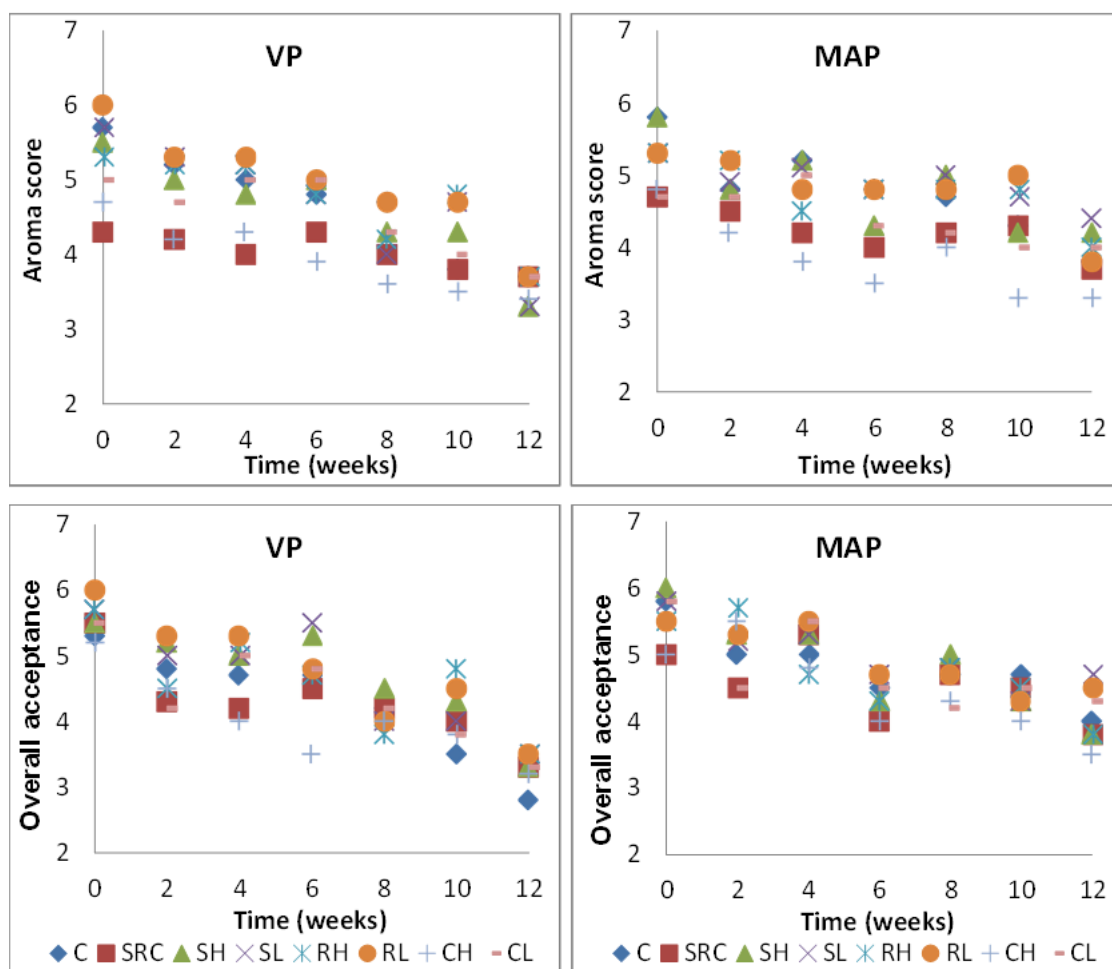


Figure 6.13: Aroma and Overall aroma score of ready to eat doner kebab stored at 4 °C under vacuum pack (VP) and modified atmosphere pack (MAP). The sensory questionnaires measured intensity on a 7-point balanced hedonic scale as follow: 7= very acceptable, 6= moderately acceptable, 5=slightly acceptable, 4= neither acceptable nor unacceptable, 3= slightly unacceptable, 2=moderately unacceptable and 1= very unacceptable.

6.4 Discussion

6.4.1 pH value

pH is a reliable indicator of food stability associated with microbial growth and chemical reactions that cause decomposition. The results indicated that pH changes significantly during storage; spice extracts addition and packaging system (Figures 6.1 and 6.2).

The pH of VP samples decreased throughout storage for all treatments. This decrease may be due to microbial action (Viuda-Martos et al. 2010a).

Pexara et al. (2002), reported a fall in pH in vacuum pack sliced turkey breast fillets and cooked pork sausages during storage at 4°C.

In MAP, the pH increased for all treatments with significant change ($P < 0.05$) starting from week 4 and until the end of the experiment. This increase in pH may be resulting from protein breakdown and production of free amino acid consequently the formation of NH_3 and amines which can be considered as compounds of alkaline reaction (Ruiz-capillas and Jimenez-Colmenero 2004). Carbohydrates and lipids reactions (Maillard reactions, lipolysis, etc.) can also be involved in pH increases. Similarly, Moore and Gill (1987) reported an increase in pH values in lamb meat product stored under MAP over storage time, attributed to tissue breakdown over time.

A significant statistical difference between VP and MAP was observed in regards to pH values for all treatments, with the lowest value obtained from a high level of sumac (SH). In contrast to this results, Viuda-Martos et al.(2010a) found that storage conditions (air pack, VP and MAP) had no significant influence on pH values of mortadella stored at 4°C during the shelf life study.

The use of high level of sumac extracts (SH) significantly reduced the pH values from time of addition (week 0) until the end of the experiment (week 12) for both

storage conditions. The lower pH values perceived from the sumac treated sample (SH) perhaps due to organic acid found in their composition represented by citric and malic acids which give sumac a sour taste (sumac pH 2.5) (Nasar-Abbas et al. 2004)

6.4.1.1 Water activity (a_w)

The change in water activity in doner kebab samples in both VP and MAP during the storage period is shown in Figures (6.3 and 6. 4). Generally, a_w values trend to gradually decrease in all samples for both packaging methods ($P < 0.05$) with a significant difference between packaging methods and treatment groups. The change in a_w during storage was probably a consequence of the loss of free water, which hardly influences the decrease in a_w . Subsequently, the loss of bound water reduced markedly the a_w value.

The a_w ranged between (0.91-0.88) in VP samples and (0.92- 0.89) in MAP samples. Leistner et al. (1981), reported that water activity has a decisive influence on the growth of micro-organisms in meat products, thereby on product shelf life. Water activity of 0.96 or less inhibits the growth of pathogenic bacteria and most spoilage microorganisms (Banwart 1979).

Water activity dropped for all doner kebab samples with added spice extracts compared with control samples. This reduction was perhaps due to high water holding capacity in spice extracts. The effect of natural extracts has been reported to effectively reduce the water activity in meat products (Fernández-López et al. 2005, Viuda-Martos et al. 2010a)

6.4.2 Microbial analysis

The changes of TVC and LAB during storage indicated a significant difference between treatments in both VP and MAP doner kebab (Figure 6.5 – 6.8). The effectiveness of cooking ($>70^{\circ}\text{C}$) and aseptic process conditions, together with the presence of the sodium chloride in the products effectively prevented the growth of enterobacteria, coliforms, moulds and yeasts, *Listeria monocytogenes* and *Salmonella spp.* during the storage time.

Aseptic conditions in preparing, processing and storing doner kebab samples were used, nevertheless cross contamination from the environment and the survival of resistant cells was possible. TVC and LAB were measured for this product; the former increased steadily regarding the storage time while the later increased in control samples only.

Satisfactory levels for TVC in ready to eat meat products are $<10^6 \log \text{CFUg}^{-1}$, and $(10^6 - < 10^8) \log \text{CFUg}^{-1}$ are borderline, while $\geq 10^8 \log \text{CFUg}^{-1}$ are considered as unsatisfactory (Health Protection Agency 2009b).

Control samples under both storage conditions reached unsatisfactory level at week 10. Whereas, spice extracts samples maintained the viable bacteria counts below the unsatisfactory level until the end of the experiment (week 12).

Spice extracts, particularly from CH, RH and SRC have shown significant inhibitory activity, while less potent activity was revealed from the other extracts. The lower microbial counts were obtained from spice extracts samples, principally due to their antimicrobial effect which contributed to the bioactive compounds present in spice composition, primarily polyphenols and terpenes (Matan et al. 2006, Viuda-Martos et al. 2008). In agreement with this result (Fernández-López et al. 2005, Zhang et al. 2009, Viuda-Martos et al. 2010a)

Fernández-López et al.(2005), stated that spice and herbs extracts namely rosemary extracts, and citrus extracts had an inhibitory effect against LAB in beef meatball stored at 8°C for 12 days. According to Zhang et al.(2009), application of a mix spice extracts of rosemary and liquorice (1:1) at different concentrations (2.5, 5, and 10 mg/ml) were significantly reduced mesophilic bacteria, lactic acid bacteria and *L. monocytogenes* in vacuum pack cooked ham stored at refrigerated temperature during storage time.

In order to predict the growth of TVC and LAB in doner kebab samples Baranyi and Roberts model was applied by using DMFit program (Table 6.1 and 6.2).. Accurate estimates for the microbial growth kinetic parameters were obtained from the DMFit model for both TVC and LAB, indicating a reliable and appropriate model used. In line with current finding the results obtained from Mataragas et al. (2006) reported that the Baranyi model was better described the data for spoilage of cooked cured meat products at 0 and 4°C.

High R^2 values resulting from the DMFit model are an indication of a good fit, as reported by Banja (2010) who stated that a better prediction is obtained from the model when the R^2 value is high ($0 < R^2 < 1$). Application of this model resulted in higher growth rate values for TVC than LAB for both packaging conditions with negative values observed from spice extracts samples in LAB only.

Lower growth rate obtained from CH and SRC for TVC and LAB in both storage conditions than other treatments. A likely explanation is that composition is linked to the high antimicrobial efficacy in CH and SRC. The reduced growth rate for LAB than TVC is probably due to an increased requirement for energy substrate for maintenance, and the negative growth rate mainly resulting from

the antibacterial effect of spice extracts against LAB (Fernández-López et al. 2005).

Not all the samples showed a lag time which probably depended on the overall metabolic rate of the cell. Robinson et al. (1998), reported that lag time in biological term represent a transition period when the bacterial cell adapt to their new environmental conditions.

The growth kinetic estimate indicated that TVC attained higher values for final cell density (y_{End}) than LAB regardless of storage time, with the higher values for y_{End} observed in the control sample.

6.4.3 Changes in thiobarbituric acid reacting substances (TBARS) of RTE doner kebab during storage

The effects of spice extracts and packaging type on secondary lipid oxidation (TBARS) of RTE doner kebab during storage time at 4°C was illustrated in (Figures 6.9, and 6.10).

Generally, storage time had a significant impact on the development of secondary lipid oxidation in RTE doner kebab, which increased during 3 months storage. As reported by Steele (2004), lipid oxidation in muscle food occurs during handling, preparation, storage, cooking and subsequently refrigerated storage, during these processes iron is released from high molecular weight source such as hemoglobin, myoglobin and ferritin and made available to low molecular weight compound (e.g. amino acid, phosphates) which is supposed to form chelates (Decker et al. 1993).

The increase in TBARS data of doner kebab samples were significantly different ($P < 0.05$) between treatments, indicating different oxidation rates (Table 6.5 and 6.6).

The results indicated that samples treated with (RH, CH and SRC) had considerably lower TBA values than the control samples in both VP and MAP RTE doner kebab for each sampling time throughout the storage period. The TBA values of RH, CH and SRC treated samples ranged between (0.34-0.63) and (0.36-0.72) in VP and MAP respectively. These outcomes suggested that RH, CH and SRC retarded lipid oxidation immediately after cooking and during storage time due to their antioxidant activity, which is a concentration dependent. Current results agree with previously reported data by (Fernández-López et al. 2003, Fernández-López et al. 2005, Doolaege et al. 2012), for the same and other type of spice extracts application in meat model.

According to Fernández-López et al (2003), rosemary and hyssop extracts inhibited lipid oxidation in cooked pork meat stored at 4°C for eight days. Furthermore, Fernández-López et al.(2005), found that the antioxidant activity of rosemary extract in cooked beef meatball is depending on rosemary types, both rosemary oil extracts and rosemary water extracts maintain the initial TBA values until the end of the experiment (12 days), whereas, rosemary oil and water miscible extracts slightly increased TBA values during the first 6 day and then become stable after that. Similarly, Doolaege et al. (2012), reported that the addition of rosemary extract at a level of (250, 500, and 750 mg/kg) during preparation of liver patties had a positive effect on retarding lipid oxidation, however their antioxidant activity was concentration independent. In this study, rosemary and cinnamon antioxidant property were concentration dependent and higher antioxidant activity observed in sample treated with high concentration of spice extracts in both packaging methods employed. On the other hand, study reported by Rojas and Brewer (2007), showed that the

addition of rosemary extract (Herbalox®) at 200 mg/kg did not retard lipid oxidation in cooked pork patties during 8 days storage at 4°C.

The antioxidant activity of RH, SRC and CH is due to the phenolic compounds present (Pérez et al. 2007, Su et al. 2007, Hossain et al. 2011) as well as flavonoids (Caillet et al. 2007, Prasad et al. 2009). All spice extracts samples showed antioxidant effect except sumac (SH and SL) which showed pro-oxidant property. The antioxidant activity of natural extracts are generally accepted (Shan et al. 2009, Viuda-Martos et al. 2010a, Kim et al. 2011) and seem to be due to the ability of spice extracts to interfere with the propagation reactions (Russo et al. 2000, Laguerre et al. 2007) or can act as the scavengers of free radicals, hydrogen donation and ion chelation (Amarowicz et al. 2004, Hinneburg et al. 2006).

The pro-oxidant effect of sumac extract could be due to high flavonoid compound in its composition. Many studies have been reported the pro-oxidant effect of flavonoid (Cao et al. 1997, Procházková et al. 2011). As reported by Viuda-Martos et al. (2010a), flavonoids act either antioxidants or pro-oxidants. the antioxidant effect of flavonoid is dependent on their structural features (Choe and Min 2009), however their pro-oxidant effect is mainly depended on concentration, temperature, light, type of substrate, and also the physical state of the system (Yanishlieva-Maslarova 2001). There is no reported data about the antioxidant or the pro-oxidant effect of sumac hydro alcoholic extract in meat products; although their antioxidant effect *in vitro* is well documented (Rayne and Mazza 2007).

The effect of packaging types (VP and MAP) on lipid oxidation of doner kebab sample were statistically different ($P < 0.05$) in all treatment groups. Lower TBARS values were obtained from VP condition compering with sample stored

under MAP condition. However, neither packaging method (VP and MAP) inhibited the oxidation process in doner kebab samples, perhaps due to the level of residual oxygen (Doherty et al. 1996, Smiddy et al. 2002). According to Smiddy et al. (2002), oxygen (residual oxygen) cannot be totally removed from vacuum pack (VP) and modified atmosphere pack (MAP), since oxygen could remain within 0–2%, which could be sufficient to cause rancidity.

Higher TBARS values were obtained from MAP samples ($P < 0.05$) than VP samples in all treatment groups, possibly due to higher levels of residual oxygen and also the ability of CO₂ to dissolve with the water of meat and the presence of oxygen can cause aerobic bacterial growth, consequently promoting lipid oxidation in MAP condition (Frank and Heather 1992).

Consistent with current findings the results obtained from (Berruga et al. 2005, Soldatou et al. 2009, Viuda-Martos et al. 2010a). As reported by Berruga et al (2005), lipid oxidation in lamb meat stored at 2 C under vacuum and three different modified atmospheres (A: 40% CO₂/60% N₂; B: 80% CO₂/20% O₂ and C: 80% CO₂/20% N₂) increased as time progress in all four groups, but was more pronounced in B (with O₂), and less in vacuum pack. Soldatou et al. (2009), reported that vacuum packaging significantly reduced lipid oxidation than modified atmosphere (30% CO₂ and 70% N₂) of Souvlaki (A Greek delicacy lamb meat product) stored at 4 C for 13 days

Prediction the oxidation rate in RTE VP and MAP doner kebab samples were obtained from linear and quadratic polynomial models (Table 6.5 and 6.6). Both models were well fitted to the TBARS data To evaluate the effectiveness of adding extra parameter to predict the oxidation rate in RTE doner kebab samples the differences between the two models were compared to the measuring error (Table 6 7) The differences were smaller than the measuring

error in VP samples, whereas in MPA samples were slightly greater. Accordingly, it is worthwhile to add the extra parameter (polynomial quadratic model) to predict the oxidation rate in MAP samples (Zwietering et al. 1990).

6.4.4 Sensory evaluation

Sensory evaluation is an important step towards demonstrating both consumer preference and the shelf life of the new products (Jaeger et al. 2011). A wide range of data was reported regarding the effect of spice extracts on the sensory evaluation of cooked meat products during the storage time (Fernández-López et al. 2003, Fernández-López et al. 2005, Viuda-Martos et al. 2010a, b). However, no reported data concerning the sensory evaluation of RTE doner kebab treated with spice extracts.

6.4.4.1 Ranking test

Regarding the sensory results for done kebab samples (Figure 6.11), ranking test (all attributes) provided useful information about the consumer preference for different treatments. It is important to notice that only spice and taste acceptability scores showed significant differences between different treatments ($P < 0.05$). For the acceptability of the taste attribute, a higher rank sum was obtained from Control, SH, SRC, CL, SM, RL, RM, SL, CM when compared with CH and RH ($P < 0.05$). Whereas, for the spiciness levels, the highest rank sum occurred in SRC, CH and CM, CL, SH, SM, Control, RH samples ($P < 0.05$). These differences could be due to the consumer discrimination or perhaps consumer not familiar with this type of spice extracts in meat product that have sweet and warm-spicy test (cinnamon) and strong, fresh, woody, herbaceous (rosemary). Although lower rank scores for test acceptability of RH, CH, CM and SL were obtained from consumer panellists, they are still within the acceptable range.

Hayouni et al. (2008), found that *Salvia. officinalis* L.(sage) and *Schinus. molle* L.(Californian pepper) essential oil had notable effects on the flavour and taste of the minced meat acceptability at concentrations more than 2% and 1.5% respectively. *S. officinalis* essential oil at concentration of >2% was unacceptable to the panellist, may be due to *S. officinalis* essential oil has a strong, warm, spicy herbaceous, and camphoraceous smell.

6.4.4.2 Hedonic test

The changes in sensory evaluation of VP and MAP control and spice extracts treated doner kebab samples stored at 4°C are presented in (Figures 6.12 and 6.13). It is important to observe that from the five attributes evaluated, only two of them (aroma and overall acceptance), displayed differences attributed to the spice extracts addition. No significant differences ($P > 0.05$) were observed from samples treated with CH, CL and SRC in both storage conditions employed, which could be due to the type of spice extracts added which potential masking other aromas in RTE doner kebab stored at refrigerated temperature. However, other treatments observed a significant difference in aroma score during storage time, probably due to the development of rancidity in high fat meat products. The significant change in aroma score started at week 8 in VP storage conditions in SH and SL samples, and at week 6 in MAP storage conditions in SH sample (Table 5.20), this could be related to the higher TBA values in these types of samples comparing with other samples. Changes in TBA values appear to display significant differences before panellist could perceive the increased rancidity. These results propose that the panellist may not be sensitive enough to detect differences of TBA values below a threshold or may be spice extracts could potential masking the rancidity. Tarladgis et al.(1960) and Jayasingh et al.(2002) reported that a limit of 1 mg

malonaldehyde/kg meats has been suggested for sensory perceived rancidity. in line with our results (Fernández-López et al. 2005) and (Mielnik et al. 2008), they found that aroma rancidity score provided useful information on the changes due to treatment and storage time in cooked meatball and cooked turkey thigh respectively.

Similar results were obtained from the overall acceptance score for all treatments in both storage conditions. This similarity is perhaps due to the effect of the aroma score on the panellist discrimination. According to Aleson-Carbonell et al. (2005), consumer acceptance is a complex process in which perceived information from foodstuffs is incorporated by discrimination and during tasting. Additionally, Platter et al. (2003), stated that small changes in odour sensory rating significantly influenced overall acceptability of steak beef meat.

As regards to storage, all attributes evaluated signified useful information to determine the shelf life of doner kebab ($P < 0.05$) which is coincided with other methods (e.g. microbial, physical and chemical analysis). For colour attribute, both storage time and conditions had significant influences on colour acceptability score, the likely explanation of that is associated with pigment oxidation and thereby metmyoglobin formation (McKenna et al. 2005). On the other hand, no significant effect was observed because of different treatment groups employed, probably addition of spice extracts at different concentrations used did not adversely affect the colour of doner kebab sample. According to Carpenter (2001), visual determinations (sensory colour evaluation) are a golden standard for evaluating different treatment groups and estimation consumer perception.

Sensory attributes, principally colour, texture, overall appearance presented significant difference between VP and MAP for all treatment groups regarding the storage time ($P < 0.05$), with high scores obtained from VP samples. These results are in line with other results obtained by Viuda-Martos et al. (2010a) who found that beef burger containing rosemary extracts and orange dietary fibre stored in vacuum packaging was the most appreciated sample compared with other packaging conditions (air packaging and MAP).

In general, although low scores were obtained from CH and SRC for aroma and overall acceptance attribute in both VP and MAP sample, nevertheless both CH and SRC are still within the acceptable level. Additionally CH under VP storage condition maintained the aroma and overall acceptance score stable until the end of the storage time.

6.5 Conclusion

The combination of spice essential oils and vacuum packaging seems to be a technologically viable alternative for elaborating RTE meat products. Spice extracts mainly cinnamon and Rosemary at high concentration and the combination of sumac; rosemary and cinnamon (SRC) have a potent effect on retarding lipid oxidation, inhibition microbial growth and extending the shelf life of RTE doner kebab. Consumers judged the meat acceptability of the product largely on the appearance of meat because consumers used discolouration as an indicator of wholesomeness and freshness, which is particularly influenced by the colour of the product. Sensory evaluation in both the ranking and hedonic tests showed that adding spice extracts does not alter the colour acceptability of RTE meat product compared to the control samples ($P > 0.05$).

Furthermore, modelling the bacterial growth by using Baranyi and Roberts's model with DMfit v2 has given a reliable estimate of growth kinetic parameter for TVC and LAB with a higher coefficient of determination, thus allowed for better prediction of the shelf life in RTE doner kebab.

Chapter 7

7 Antimicrobial activity of spice extracts application to modified atmosphere packaged ready to eat doner kebab (storage at 4°C) in a challenge test against *Listeria monocytogenes*

7.1 Introduction

Listeria monocytogenes is one of the most important psychrotrophic food pathogens related to an aerobically packed cooked meat products is considered as a biological hazard of particular interest to food processors (Nesbakken et al. 1996, Lado and Yousef 2007). This bacterium is widely distributed in nature in soil, plants, animals, water, dust, as well as in different food processing and storage environments (Farber and Peterkin 2000).

The pathogen can cross-contaminate food contact surfaces, equipment, floors, drains, and employees (Beresford et al. 2001). Moreover, *L. monocytogenes* has the ability to grow in wet conditions and form biofilms in the processing environment that are hard to eliminate during cleaning and sanitizing (Borucki et al. 2003, Oliveira et al. 2010, Hereu et al. 2001), it also has the ability to tolerate heat, salt and grow at refrigeration temperatures and survive freezing (McClure et al. 1997, Hill et al. 2002, Liu et al. 2002).

Listeria monocytogenes as a food-borne pathogen has substantial public health and economic impacts. The infection of humans may result in severe clinical disease (listeriosis) with high mortality, the disease can exist in two forms, including a gastrointestinal illness and invasive listeriosis which can be life threatening (Posfay-Barbe and Wald 2004). It particularly affects people with reduced immunity (immune-compromised), which include elderly,

pregnant women and new-born infants (Brachman et al. 2009, Sasakawa et al. 2009). A number of studies have been reported that most Ready to eat (RTE) meat producers were considered as a main source for *Listeria* infection (Rez et al. 2007, Ross et al. 2009, Mejlholm et al. 2010). Since, RTE cooked meats products are consumed without further heating and have a long shelf-life, contamination by *L. monocytogenes* of RTE cooked meat products is considered as a major safety concern (USDA-FSIS 2003, Zhu et al. 2005).

According to the Food Standards Agency (2005) in the UK, RTE products are classified in different categories, depending on whether or not they are able to sustain growth of *L. monocytogenes*. This condition establishes that *L. monocytogenes* should not exceed the limit of 100 CFUg⁻¹ in RTE product throughout the shelf life, Cooked-doner kebab are included in this condition; consequently, they should comply with the criteria, regardless of their packaging (vacuum-packed, modified atmosphere packed or not packed).

To our knowledge, a quantitative assessment of the safety (especially against *L. Monocytogenes*) in RTE doner kebab under combined effect of spice addition and modified atmosphere packaging has not been studied previously. Therefore, the aims of this study are to determine the effectiveness of antibacterial culinary spice extracts applied to modified atmosphere packed ready to eat doner kebab challenged with *L. monocytogenes* and stored at 4±1°C and to compare the effectiveness of two application methods used.

7.2 Materials and methods

7.2.1 Bacterial culture preparation

Two strains of *Listeria monocytogenes* NCTC5105 (human isolate) and NCTC7973 (type strain) were used. Working stock cultures were prepared by overnight incubation in brain heart infusion broth (BHI) at 37°C to reach 10^9 CFUml⁻¹ after 24 h incubation. The culture was standardized by diluting to 10^7 CFUml⁻¹. Miles and Misra colony count method was used to verify the bacterial level (Heritage et al. 2000). Subsequently, the inoculum was prepared by mixing equal volumes of each culture and dispensing in a sterile Eppendorf tubes to be used for the challenge test.

7.2.2 Natural extracts

Rosemary and cinnamon oleoresins were kindly donated By Kalsec as detailed in Section 3.1. Sumac was obtained from Green Cuisine Food Products and hydro alcoholic extracts of the sumac was prepared as detailed in Section 3.1.1.2.

7.2.3 Experimental design

Two experiments were designed following a challenge testing protocol against *Listeria monocytogenes* in MAP ready to eat doner kebab, to determine the antibacterial activity of spices extract when applied to RTE doner kebab slice and modified atmosphere packaged storage at 4°±1C.

7.2.4 Fate of pathogen on ready to eat doner kebab mixed with spice extracts before cooking

7.2.4.1 Product formulation

Doner kebab was manufactured in the food and nutrition lab as in Section 3.10.2. The kebab was subjected to different treatments (spice adding, cooking, and packaging). A batch of 4 kg doner kebab was thawed and divided into the 8

blocks of about 500g and randomly assigned to 8 treatments: (Rosemary oleoresin, sumac alcoholic extract and cinnamon oleoresin) at two concentrations (0.05 and 0.2 ml/100 g) and combination of (1:1:1) mixture of rosemary, cinnamon and sumac (high concentration level); while one block was to be left without spices as a control.

7.2.4.2 Product processing

The product and samples were prepared in a pilot plant resembling commercial processing conditions. All ingredients were homogenized in a bowl mixer with a spiral dough hook (Silverson, Birmingham, UK) for 5 min. For each treatment, the corresponding extracts were added at the concentrations mentioned above, and then mixed again for 5 min. kebab batches were formed and cooked as detailed in Section 3.11. When the endpoint temperature was achieved, the kebab slices were cooled down immediately by using ice bath to reach a product temperature below 12°C. The freshly cooked slices (35g) were packaged using a modified atmosphere packaging system as details in Section 3.12. To inoculated the product aseptically a sterile Eppendorf containing (0.35 ml, 10^7 CFUml⁻¹) *L. monocytogenes* was deposited in to each pack before sealing then the Eppendorf was opened to allow the bacteria to distributed equally on the kebab slice using massaged with fingers to obtain level of 10^5 CFUg⁻¹. The slices were stored in a cooler $4\pm1^\circ\text{C}$ (Williams Refrigeration and Air-conditioning, installation by general refrigeration, Limited). 96 *L. monocytogenes* inoculated packages were made for this application method giving 12 packages for each treatment.

7.2.5 Inactivation of pathogen after cooking

Doner kebab slices were prepared and cooked as mentioned above but without addition of spice extracts.

Freshly cooked doner kebab samples were divided in to 8 batches, seven were treated with spice extracts at two concentrations; (Rosemary oleoresin, sumac alcoholic extract and cinnamon oleoresin, and a combination of high concentration of equal volume of these spices were used) by dipping the slices for one second into the spice preparation. The level of spice extracts were 0.025 and 0.05 ml/100g , which were mixed with sunflower oil, 1 part of spice extract diluted with 1.5 part of sunflower oil and 1 part of spice extract diluted with 4 parts of sunflower oil respectively.

Treatment and control samples were prepared, inoculated and stored as mentioned above. 96 *L. monocytogenes* inoculated packages were made for this application method giving 12 packages for each treatment. Samples were taken for microbiological analysis as mention below.

7.2.6 Microbiological analysis

Samples were taken for microbiological analysis at day 1, 5, 7, 14, 21 and 28; a minimum of 2 packages were used for each treatment. Packages were surface wiped on the outside with a paper towel soaked in 70% (vol/ vol) ethanol to prevent contamination. *L. monocytogenes* presence or absence and numeration were determined as detailed in Chapter 3, Section 3.9. according to standard methodologies (Health Protection Agency 2009a), a composite of 25 g cooked doner kebab sample was formed with portions of at least 3 doner kebab slices was required to determine the presence/absence of *L. monocytogenes*.

For LAB and TVC, serial dilution of aliquots were plated out As detailed in Section 3.13.1. Replicate of three appropriate dilutions (three plat per dilution) on every test time were numerated. Bacterial counts were transformed into logarithms from the number of colony forming units (CFUg⁻¹) of RTE doner kebab before being statistically analysed.

7.2.7 Statistical analysis

A multilevel Factorial design analysis with ANOVA, GLM was used to compare different treatment groups as in Section 3.14. The organism decline slope, correlation coefficient and regression for *Listeria monocytogenes*, TVC for each treatment were calculated from linear regressions using Excel (Excel, 2010)

7.3 Results

7.3.1 Survival of *L. monocytogenes* on ready to eat doner kebab mixed with spice extracts before cooking.

Listeria monocytogenes, Total viable counts (TVC), and lactic acid bacteria (LAB) for modified atmosphere RTE doner kebab slices treated with or without spice extracts during storage at 4±1°C, are illustrated in (Figures 7.1, 7. 2 and Table 7.1).

For *L. monocytogenes*, the results indicated that both storage time and treatment influenced the growth of *L. monocytogenes* in RTE doner kebab. The ANOVA reflected statistical differences in bacterial counts regarding storage time and treatment ($P < 0.001$). The control sample exhibited the greatest growth, the initial *L. monocytogenes* population was 5 log₁₀ CFUg⁻¹; the microbial count increased steadily during storage time, reaching 7.8 log₁₀ CFUg⁻¹ after 28 days of refrigerated storage ($P < 0.05$). RTE doner kebab slices treated with spice extracts demonstrated different efficacy against *L. monocytogenes*. There was a trend of increase in bacterial population for RH,

RL and SL treated samples. In contrast SRC, SH and CL treated samples slightly decreased the bacterial population during storage ($P > 0.05$). CH presented high efficacy against *L. monocytogenes*, which decreased from 5 log₁₀ at day 1 to 3.5 log₁₀ CFUg⁻¹ after 28 days of storage ($P < 0.05$) (Figure 7.1).

The treatment with CH decreased the bacterial count by 0.22, 1, 1.8, 2.1, and 4.4 log₁₀ CFUg⁻¹ on days 1, 5, 7, 14, 21 and 28 when compared with control sample ($P < 0.05$). The higher concentration of cinnamon oleoresin gave the greatest inhibition of *L. monocytogenes*. Linear regression was used to determine the rate of growth or decline in bacterial population at the end of storage (Table 7.2). The outcomes from the linear regression indicated that while higher growth was obtained from control sample with $R^2 = 0.86$, a higher rate of decline was obtained from CH treated sample ($R^2: 0.94$). The antimicrobial efficacy of RTE doner kebab treated with spice extracts was in the following order: CH > SRC > CL > SH > SL > RH > RL.

For TVC, control samples showed a steady increase in bacterial population with storage time, the significant increase started at day 14 of the storage and the major increase occurred at day 28 ($P < 0.05$). For spice extract treated samples, the same scenario for *L. monocytogenes* were happen against TVC with some variation in antimicrobial efficacy. Higher antimicrobial effects were obtained from sample treated with CH. The total viable count for RH, RL and SL increased gradually regarding storage time, the ANOVA test indicated that significant statistical differences started from day 14 and continued until the end of the storage. For SRC, SH and CL the bacterial population remained stable until the end of the experiment (Figure 7.2). The antimicrobial effect of spice extracts against TVC followed this ascending order CH > SRC = CL = SH > SL = RH > RL (Table 7.3).

Incorporation spice extracts in the formulation of RTE doner kebab, expressed a different levels of inhibition against LAB relative to the control sample ($P > 0.05$). For the control sample LAB remain at the same level until the end of the experiment, while for spice extract samples; LAB counts decreased and reached the not detected limit at different day (Table 7.1).

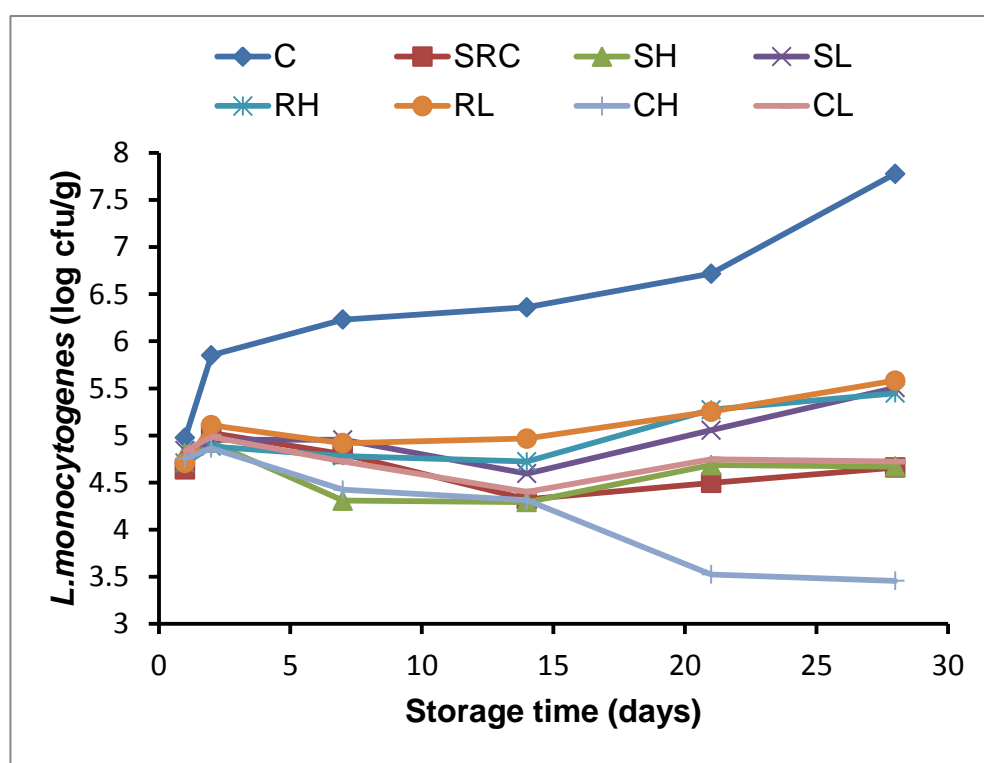


Figure 7.1: Numeration of *L. monocytogenes* in ready to eat doner kebab treated with spice extracts before cooking. C: control sample, SRC: combination of sumac, rosemary and cinnamon, SH: sumac at high level, SL, sumac at low level, RH: rosemary at high level, RL: rosemary at low level, CH: cinnamon at high level, CL: cinnamon at low level. LSD: 0.26 at 5%

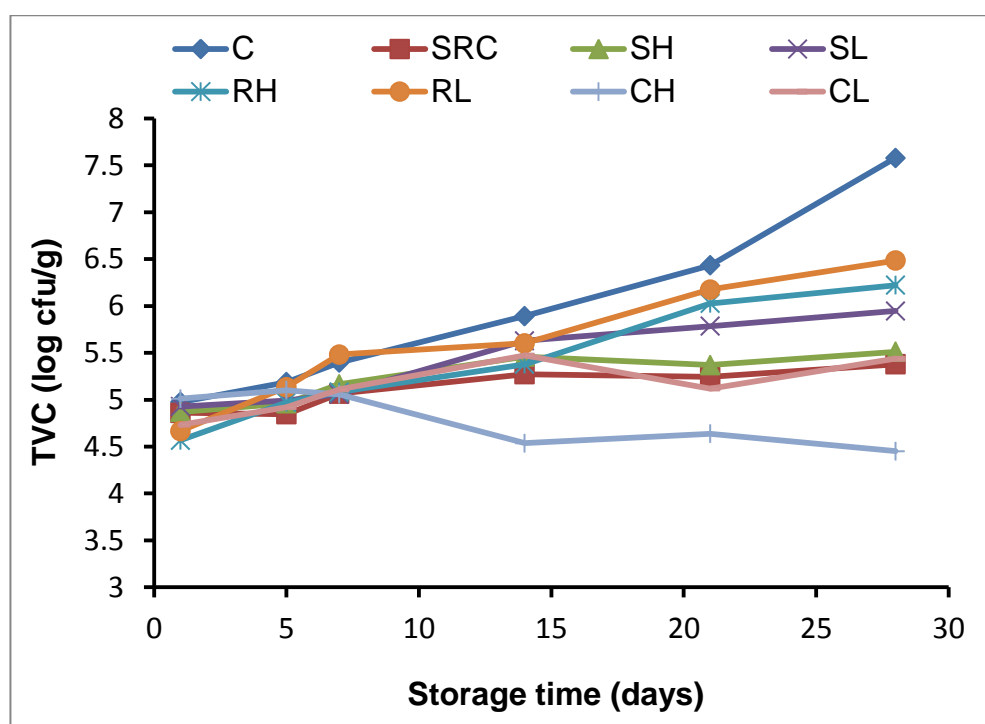


Figure 7.2: Numeration of TVC in ready to eat doner kebab treated with spice extract before cooking. LSD: 0.8 at 5%. For sample key see Figure. 7.1.

Table 7.1: Lactic acid bacteria (LAB) counts (log CFUml⁻¹) in RTE doner kebab during storage time with added natural extracts were applied before and after cooking.

| Method for application | Time (days) | Treatments | | | | | | | |
|------------------------|-------------|-------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | | Control | SRC | SH | SL | RH | RL | CH | CL |
| BC* | 1 | 4.4 ^{ax} | 3.6 ^{ax} | 3.6 ^{ax} | 3.8 ^{ax} | 3.7 ^{ax} | 3.7 ^{ax} | 3.8 ^{ax} | 3.9 ^{ax} |
| | 5 | 4.2 ^{ax} | 3.5 ^{ax} | 3.7 ^{ax} | 3.8 ^{ax} | 3.6 ^{ax} | 3.6 ^{ax} | 3.3 ^{ax} | 3.5 ^{ax} |
| | 7 | 4.3 ^{ax} | 1.8 ^{byz} | 2.6 ^{ayz} | 2.7 ^{axz} | n.d ^{byz} | n.d ^{byz} | n.d ^{byz} | n.d ^{byz} |
| | 14 | 3.8 ^{ax} | n.d ^{byz} | 2.5 ^{axz} | 2.6 ^{axz} | n.d ^{byz} | n.d ^{byz} | n.d ^{byz} | n.d ^{byz} |
| | 21 | 3.9 ^{ax} | n.d ^{by} | n.d ^{by} | 1.8 ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} |
| | 28 | 4.3 ^{ax} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} |
| AC* | 1 | 4.8 ^{ax} | 3.8 ^{ax} | 3.6 ^{ax} | 3.7 ^{ax} | 3.5 ^{ax} | 3.8 ^{ax} | 3.3 ^{ax} | 3.5 ^{ax} |
| | 5 | 4.6 ^{ax} | 3.6 ^{ax} | 3.4 ^{ax} | 3.5 ^{ax} | 3.3 ^{ax} | 3.6 ^{ax} | 3.2 ^{ax} | 3.3 ^{ax} |
| | 7 | 4.3 ^{ax} | 2.9 ^{axz} | 2.2 ^{byz} | 2.5 ^{ayz} | 1.8 ^{byz} | n.d ^{byz} | n.d ^{byz} | n.d ^{byz} |
| | 14 | 3.6 ^{ax} | 2.4 ^{ay} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} |
| | 21 | 3.4 ^{ax} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} |
| | 28 | 3.3 ^{ax} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} | n.d ^{by} |

* BC: before cooking, AC: after cooking; n.d: not detected (<1.69 log detection limit), ^{(a-}

^{b)} Means within a column with different letters are significantly different ($P < 0.05$), ^(x-z)

Means within a row with different letters are significantly different ($P < 0.05$), LSD: 1.72 at 5%, for sample key see Figure 7.1.

7.3.2 Inactivation of pathogen after cooking

The growth of *L. monocytogenes*, TVC and LAB on refrigerated ($4\pm1^{\circ}\text{C}$) modified atmosphere pack RTE doner kebab with or without spice extracts during storage are shown in (Figures 7.3 and 7. 4) and (Table 7.1).

The initial level of *L. monocytogenes* for control samples was $5 \log_{10} \text{CFUg}^{-1}$, after 28 days storage the level was increased to reach $7.5 \log_{10} \text{CFUg}^{-1}$ ($P < 0.05$). For spice, all samples showed a significant inhibitory effect ($P < 0.05$) with different efficacy compared with control and the reduction started from the first day. Cinnamon oleoresin at high concentration exhibited the greatest antibacterial effect; the initial bacterial count was $4.3 \log_{10} \text{CFUg}^{-1}$ at day 1 which decreased to reach the not detected limit at day 14. The rest of spice extract the level of *L. monocytogenes* was suppressed (Figure 7.3).

The ANOVA showed that TVC was significantly affected ($P < 0.05$) by both the storage period and treatments. While, the bacterial population decreased in spice treated samples, the control exhibited a significant increase in TVC over time (Figure 7.4).

SRC, SH, SL, RH, RL, and CL slightly decreased the TVC than the initial level, and then the level remained stable until the end of the experiment. However, CH showed a sharp decrease in the TVC over time. The growth or the decline rates obtained from the linear regression were presented at (Table 7.3).

For LAB, all samples with or without spice extract application shown a decline in LAB count. In control samples, LAB decreased from $4.8 \log_{10} \text{CFUml}^{-1}$ at day 1 to reach $3.3 \log_{10}$ at the end of the storage ($P > 0.05$); for spice treated samples LAB decreased gradually to reached the not detected limit at day 7 for CH, CL and day 14 for RH, RL, SH, SL and day 21 for SRC ($P < 0.05$) (Table 7.1).

Overall results obtained from ANOVA (GLM) indicated that, while, both *L. monocytogenes* and TVC were significantly affected by times, treatments, application methods and the interaction between these factors ($P < 0.05$), LAB were affected by storage time only.

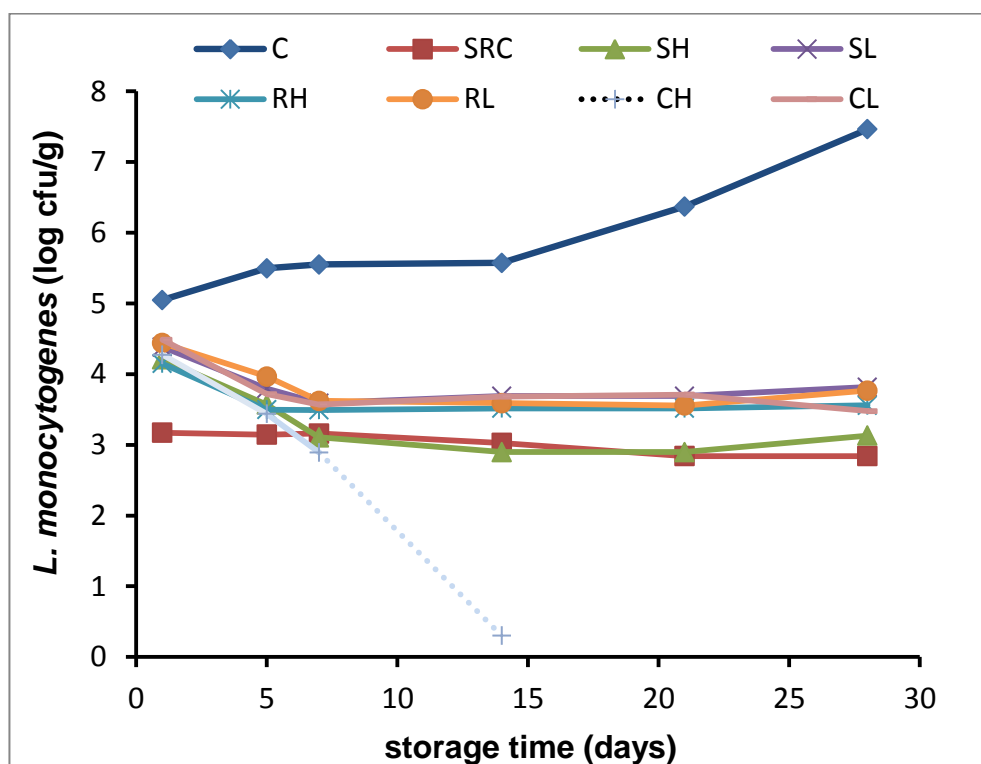


Figure 7.3: Numeration of *L. monocytogenes* in ready to eat doner kebab treated with spice extracts after cooking. LSD: 0.26 at 5 %. For sample key, see Figure 7.1.

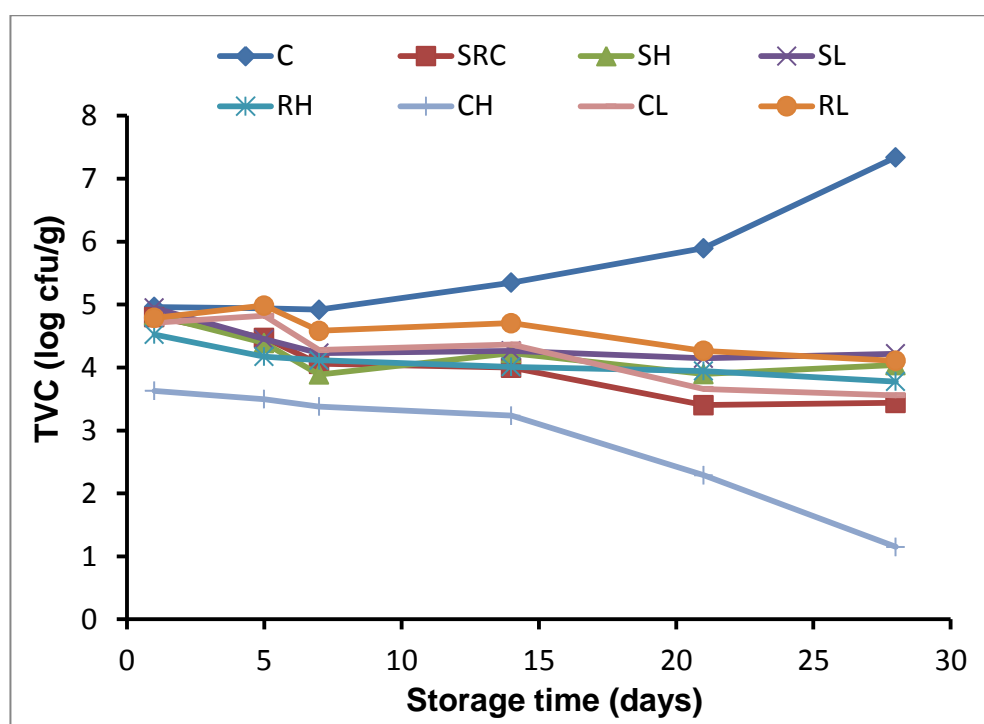


Figure 7. 4: Numeration of TVC in ready to eat doner kebab treated with spice extracts after cooking. LSD: 0.8 at 5%. For sample key, see Figure 7.1.

Table 7.2: Pathogen growth rate after 28 days of storage for *L. monocytogenes* obtained from linear regression with regression parameter.

| Spice addition | Type of bacteria | Treatment | Growth Rate (μ_{\max} day ⁻¹)* | Polynomial Linear (y= a + b*x) | | |
|----------------|-------------------------|-----------|---|--------------------------------|--------|------------------|
| | | | | a** | b** | R ^{2**} |
| BC | <i>L. monocytogenes</i> | Control | 0.100 | 5.34 | 0.079 | 0.86 |
| | | SRC | -0.001 | 4.78 | -0.008 | 0.59 |
| | | SH | -0.005 | 4.84 | -0.010 | 0.33 |
| | | SL | 0.024 | 4.77 | 0.018 | 0.40 |
| | | RH | 0.026 | 4.67 | 0.025 | 0.73 |
| | | RL | 0.031 | 4.80 | 0.027 | 0.93 |
| | | CH | -0.046 | 4.88 | -0.054 | 0.94 |
| | | CL | -0.004 | 4.83 | -0.013 | 0.34 |
| AC | <i>L. monocytogenes</i> | Control | 0.086 | 4.909 | 0.080 | 0.89 |
| | | SRC | -0.005 | 3.22 | -0.015 | 0.93 |
| | | SH | -0.039 | 3.73 | -0.034 | 0.47 |
| | | SL | -0.020 | 3.98 | -0.012 | 0.19 |
| | | RH | -0.021 | 3.78 | -0.012 | 0.23 |
| | | RL | -0.024 | 4.08 | -0.020 | 0.37 |
| | | CH | -0.142 | 4.83 | -0.312 | 0.98 |
| | | CL | -0.028 | 4.06 | -0.022 | 0.41 |

*: growth or decline rate, a**: intercept, b**: regression, R^{2**}: coefficient of determination. For sample key see Figure 7.1.

Table 7.3: Pathogen growth rate after 28 days of storage for TVC obtained from linear regression with regression parameter.

| Spice addition | Type of bacteria | Treatment | Growth Rate (μ_{\max} day ⁻¹) * | Polynomial Linear (y= a + b*x) | | |
|----------------|------------------|-----------|--|--------------------------------|-------|------------------|
| | | | | a** | b** | R ^{2**} |
| BC | TVC | Control | 0.093 | 4.74 | 0.092 | 0.97 |
| | | SRC | 0.018 | 4.95 | 0.013 | 0.85 |
| | | SH | 0.023 | 5.03 | 0.015 | 0.81 |
| | | SL | 0.036 | 4.93 | 0.023 | 0.94 |
| | | RH | 0.059 | 4.60 | 0.061 | 0.97 |
| | | RL | 0.065 | 4.86 | 0.042 | 0.93 |
| | | CH | -0.020 | 4.86 | -0.01 | 0.11 |
| | | CL | 0.025 | 4.86 | 0.021 | 0.59 |
| AC | TVC | Control | 0.085 | 4.49 | 0.085 | 0.85 |
| | | SRC | -0.049 | 4.67 | -0.05 | 0.88 |
| | | SH | -0.028 | 4.50 | -0.02 | 0.42 |
| | | SL | -0.026 | 4.63 | -0.02 | 0.50 |
| | | RH | -0.020 | 4.37 | -0.02 | 0.83 |
| | | CH | -0.031 | 4.93 | -0.03 | 0.80 |
| | | CL | -0.089 | 3.99 | -0.09 | 0.89 |

*: growth or decline rate, a**: intercept, b**: regression, R^{2**}: coefficient of determination, for sample key see Figure 7.1.

7.4 Discussion

L. monocytogenes represent a real threat resulting from post-process contamination in ready-to-eat meat products; a wide range of RTE meat products provides a suitable medium for *L. monocytogenes* multiplication (Beumer et al. 1996, Devlieghere et al. 2001, Uyttendaele et al. 2004, Asma et al. 2009).

The protocol issued by AFSSA (2001) suggested two strains of *L. monocytogenes* were used separately, a reference strain and a food isolate in a challenge test for RTE meat products. The reference strain is recommended because it is likely to assess the challenge test outcome in diverse settings. However, food isolate represents the natural contamination model. In addition, *L. monocytogenes* of 10^3 CFUml⁻¹ was the recommended inocula in a challenge test for RTE meat products. On the other hand, Lubert et al. (2011) suggested for a challenge test an inocula of 1-100 CFU g⁻¹ must be used to measure the potential growth of *L. monocytogenes* and the impact of microbial competition, besides the strains use should be typical to those found in the product being assessed and should involve pathogenic strains and those resistant to the environmental stress during the storage time. In our study the option was taken to use mixed inocula for two strains of *L. monocytogenes* NCTC5105 (human isolate) and NCTC7973 (type culture) at a level of 10^5 CFUml⁻¹. Mixture of strains reduces the variation in growth and survival that may occur under various growth conditions (Uyttendaele et al. 2004). Additionally, starting with a high inocula level would allow levels to be measured above the detection limit, at early stages of the storage time.

In this work, a challenge test protocol was carried out against *L. monocytogenes* in RTE doner kebab under the combined effect of modified

atmosphere packaging and spice extracts treatment. Recently, some studies have revealed successful applications of spice extracts with other preservation methods in order to reduce or control spoilage and pathogenic bacteria in meat products (Mytle et al. 2006, Ghalfi et al. 2007).

Two application methods for spice extracts treatment were used (application of spice extracts before cooking (BC) and after cooking (AC)) to compare the effectiveness of these application methods against *L. monocytogenes*, TVC and LAB. Current results showed that both *L. monocytogenes* and TVC were significantly affected by application methods and a higher inactivation achieved when the spice extract was applied AC (Figures 7.1 – 7.4) and (Tables 7.2 and 7.3). However, no significant effect against LAB was observed (Table 7.1). The higher antimicrobial effect occurred when spice extracts were applied AC, probably due to the following factors: firstly, the concentration of in spice on the surface of doner kebab sample was higher when spice extracts added AC than BC. Secondly, the ability of spice extracts to form a protective layer against bacterial group when applied on the surface of doner kebab slice (AC). Previous studies have reported that major listeriosis outbreaks associated with the contamination of RTE meat produces are often linked to cross-contamination from surfaces of equipment during processing, production or retail (Fenlon et al. 1996, Van Coillie et al. 2004 , Lin et al. 2006, Wilks et al. 2006). The spice extracts directly attack *L. monocytogenes* by different mechanisms: interaction with protein to alter their conformation, direct effect on the membrane integrity of bacteria and induced depletion of the intracellular ATP concentration (Oussalah et al. 2006) or disrupting enzyme systems (Burt et al. 2007) and compromising the genetic material of bacteria (Lanciotti et al. 2003).

Another possible explanation for higher antimicrobial activity when spice extracts are applied on the surface of the slice, which is meat fat was less likely to form protective coating around bacteria. As suggested by Cutter (2000) fat in food could form a protective coat around microbes. Additionally, Larson et al.(1996) reported that meat and meat products are a complex growth environment and may possibly provide microbial cells with excessive protection from antimicrobial agents when spice extracts were added before cooking. Finally, some of spice extract could be lost during cooking as the fat melts, particularly if the extract is fat-soluble.

The results achieved in this investigation permitted to point out the important role of spice extracts in the control of *L. monocytogenes*, TVC and LAB with various degrees of antimicrobial efficacy in modified atmosphere pack RTE meat products stored at 4 °C (Figures 7. 1– 7.4) and (Tables 7.2 and 7.3).

Cinnamon oleoresin at high concentration (CH) exhibited the strongest antimicrobial activities against *L. monocytogenes*, TVC and LAB bacteria. The inhibitory effect was less pronounced at lower concentration (CL), however still present. The antimicrobial activity of cinnamon could be due to its rich source of polyphenolic compounds represented by eugenol, cinnamaldehyde and proanthocyanidins (Lis-Balchin and Deans 1997, Baratta et al. 1998, Friedman et al. 2002, Prabuseenivasan et al. 2006, Shan et al. 2007a). Mahfuzul Hoque et al. (2008) found that cinnamon essential oil at 5% concentration, reduced *L. monocytogenes* in ground chicken meat by 2.0 log CFU /g within 1 day and with only slight reductions in cell population throughout the 15 days incubation period, this represents a bacteriostatic effect. In this results cinnamon oleoresin also showed a bacteriostatic effect against *L. monocytogenes* and a promising

bactericidal effect when high concentration (CH) was applied on the surface of the products (AC).

Greater antimicrobial effects of combined extracts (SRC) were observed against *L. monocytogenes*, TVC compared with using rosemary (RH) only, although less concentration was used. The possible explanation of that could be resulting from the synergistic actions of specific compounds present in the mixed spices. Similar results were reported by (Mau et al. 2000, Periago et al. 2001, Rota et al. 2008, Zhang et al. 2009). Mixed extracts of Chinese chive, cinnamon and corni fructus not only prevented a wide range of common food-borne pathogens but also showed excellent stability to heat, pH and storage time (Mau et al. 2000). Furthermore, Zhang et al. (2009) reported a strong potential antimicrobial effect of mixed rosemary and liquorice extracts against *L. monocytogenes* as well as several meat spoilage bacteria in fresh pork and ham products.

Sumac (SH, SL) applied following two different methods (AC, BC) showed a bacteriostatic effect against *L. monocytogenes* and TVC. However they a bactericidal against LAB in RTE modified atmosphere pack doner kebab. The antibacterial activity of sumac is particularly linked to the phenolic compounds especially Gallic acid, which possesses antimicrobial activity against several types of bacteria (Gulmez et al. 2006). Results previously discussed in Chapter 4 indicated that sumac alcoholic extracts exhibited strong antimicrobial activity against nine strains of pathogenic and spoilage bacteria including *L. monocytogenes in vitro* (Al-Kutby et al. 2009). Nasar-Abbas et al (2004) reported that an alcoholic extract of sumac had potent antibacterial activity against most food-borne pathogens (*Bacillus subtilis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.*). this was attributed to the decontamination effect

of sumac extracts against psychrotrophic, mesophilic, Enterobacteriaceae, and coliform bacteria in a broiler wing mode (Gulmez et al. 2006).

Rosemary extract (RH, RL) effectively inhibited the growth of *L. monocytogenes*, TVC and LAB in RTE doner kebab compared with the control.

Many studies have been reported on the antibacterial effects of rosemary against pathogenic and spoilage bacteria (Del et al. 2000, Djenane et al. 2003, Fernández-López et al. 2005, Rižnar et al. 2006, Gachkar et al. 2007, Zhang et al. 2009). This antibacterial activity is mostly due to the carsonic acid present in rosemary (Campo et al. 2003, Oluwatuyi et al. 2004, Bozin et al. 2007, Gachkar et al. 2007). The effect of spice extracts, application methods and storage time on RTE modified atmosphere packaged doner kebab showed the same trend against *L. monocytogenes* and TVC with a little variation. In this investigation, LAB was detected in RTE doner kebab for both control and spice extract treated samples (Table 6.1). Cross contamination from the environment as well as survival of spore resistant cells was possible, as it is in commercial processes (Kuri et al. 1998, Fernández-López et al. 2005). In the control sample LAB was detected in both application methods, however the spoilage level was not achieved. The explanation of that is perhaps due to absence of a sufficient amount of sugar in our product. Thus, some LAB may be present but their growth on the product is controlled under storage conditions (Fernández-López et al. 2005). For spice extracts, LAB was detected from day1 then decreased to reach the not detected limit at different day of storage time. This was mostly due to antimicrobial effect of the extracts. This antimicrobial activity of spice extracts against LAB in meat and meat products has been revealed by several authors (Fernández-López et al. 2005, Zhang et al. 2009).

7.5 Conclusions

A challenge test protocol against the growth of *L. monocytogenes* demonstrated that spice extracts could be useful to control the risk associated with RTE meat products for Listeriosis. This study showed that extracts of common food spices (cinnamon, rosemary, sumac and their combination) have antibacterial effects against *L. monocytogenes*, TVC and LAB. All spice extracts had bacteriostatic effects against *L. monocytogenes*, TVC, whereas, cinnamon oleoresin at high concentration demonstrates a bactericidal effect against *L. monocytogenes* when applied on the surface of the doner kebab sample. Additionally, bactericidal effect of spice extracts were observed against LAB for both application methods used. The present data indicated that highly significant antimicrobial effect against *L. monocytogenes* and TVC could be obtained when spice extracts are applied after cooking. Cross contamination results in low levels of bacterial population ($<10^2$), thus the use of spice extracts in meat products could maintain the bacterial population below the infectious dose, becoming an additional hurdle for risk reduction; especially spice extracts are widely used in the food industry and are generally regarded as safe (GRAS). Thus, they may be considered as natural preservatives acceptable by the meat products.

Chapter 8

8 Bacterial safety of RTE doner kebab, heating and processes lethality: thermal inactivation of spore-formers increases with spice addition

8.1 Introduction

Thermal processing of meat products enhances their safety and palatability. Inadequate thermal processing can result in the presence of food-borne pathogens in cooked ready-to-eat (RTE) meat products. Although, integrated time-temperature control during cooking should be adequate to destroy vegetative bacterial pathogens, bacterial spores may possibly survive cooking and multiply during cooling (Frazier and Westhoff 1978).

Clostridium perfringens and *Bacillus cereus* are common spore forming pathogens, which cause concern to the foodservice industry (Brown 2000). Both are Gram positive spore-forming, rod shaped and ubiquitous in nature (Curtis et al. 2003, Juneja et al. 2003). Food poisoning associated with these types of pathogen is due to the ability of these bacteria to form heat-resistant spores that survive commercial cooking processes. In addition, *C. perfringens* has the ability to grow rapidly at relatively high temperatures with reported generation times as short as 8.5 min. It is well documented that *C. perfringens* and *B. cereus* can grow in a range temperatures from 6 to 52.3 °C (Johnson 1990, Juneja et al. 1999) and 4 to 55 °C respectively (Food Safety and Inspection Service and United States Department of Agriculture 2003). These pathogens are found in meat and meat products (Roberts et al. 1995, Doyle 2002) especially when prepared in large quantities (large gatherings or institutional

settings) and when not adequately cooled or its temperature has been abused for long time during post-process, handling, or storage (Bean and Griffin 1990). Unsafe levels of *C. perfringens* and *B. cereus* vegetative cells can be found in precooked, ready-to-eat meat products resulting from spore germination and enterotoxin production (Granum and Lund 1997). Thus, to keep the product safe, sufficient heating must be used to kill the bacteria before consumption. However, inadequate reheating of such products before consumption would lead to food poisoning.

The enterotoxins of *B. cereus* are produced in the food (Kramer 1989, Granum and Lund 1997, Bennett and Belay 2003), while *C. perfringens* enterotoxin (CPE) is produced during sporulation (Duncan 1973, Juneja et al. 1999). According to Collie & McClane (1998), CPE gene associated with a non-food-poisoning is commonly located on a plasmid, whereas the food-poisoning CPE gene is commonly located on the chromosome. Sarker et al. (2000), reported that *C. perfringens* strains which carry the CPE gene on the chromosome were found to be 60 times higher in heat resistant strains compared with non-food-poisoning strains.

C. perfringens was found to be the greatest food poisoning risk to public health associated with doner kebab in the UK (Gordon et al. 1995). There is a lack of information about the temperature profile, process lethality and the thermal inactivation of *C. perfringens* and *B. cereus* in lamb doner kebab. In addition, there is no reported data about the combined effect of heat treatment and spice extract application against spore forming bacteria in this product.

Thus, this study aimed to: First: investigate the thermal inactivation of spore forming bacteria (*Bacillus cereus* and *Clostridium perfringens* vegetative cells) in RTE doner kebab and evaluate the antimicrobial effectiveness of spice

extract. Second: determine the temperature profile and the process lethality of doner kebab.

8.2 Materials and methods

Experiment 1: thermal inactivation of spore forming bacteria using spice extracts.

8.2.1 *Bacterial cultures*

Culture collection strains of *Bacillus cereus* NCIMB 11925 (*Bacillus cereus* Frankland and Frankland, extensively used in thermal inactivation studies) and *Clostridium perfringens* NCTC 8239 (type strain) were obtained from the bacterial culture collection as in Section 3.2.

B. cereus and *C. perfringens* were selected because these microorganisms are considered the most common pathogenic spore-forming bacteria in ready to eat cooked meat products.

8.2.2 *Natural extracts*

Sumac was obtained from Green Cuisine Food Products as in Section 3.1 and hydro alcoholic extract was prepared as detailed in Section 3.1.1.2. While, rosemary and cinnamon oleoresin were provided by Kalsec as detailed in Section 3.1.

8.2.3 *Inoculum preparation*

B. cereus and *C. perfringens* strain were inoculated into 20 ml of brain–heart infusion broth (BHI, Oxoid Ltd., Basingstoke, and Hampshire, UK). The former was incubated aerobically (Swallow Incubators, LTE Scientific Ltd, Green field, Oldham, UK) at 30°C for 24h while, the latter was incubated in an anaerobic incubator (Modular Atmosphere Controlled System, Don Whitley Scientific

Limited, West Yorkshire, UK) at 37°C for 24h. Each culture was centrifuged at 4000g for 10 min (Rotina 46 centrifuge, Hettich Zentrifugen, Germany). The recovered cell pellets were washed three times and finally re-suspended in 10 ml of MRD. To confirm that each strain had not sporulated, spore stain (Schaeffer and Fulton's Method) was used (Health Protection Agency 2007). The concentration of a 10^7 CFU ml⁻¹ for each vegetative cell was adjusted by diluting with MRD. Plate technique were carried out to confirm the concentrations level of each bacteria as mentioned below: *B. cereus* and *C. perfringens* were serially diluted and plated onto a non-selective media, nutrient agar (NA) (Oxoid Ltd., Basingstoke, Hampshire, UK) and aerobically incubated at 30°C for 24 h (Byrne et al. 2006) and anaerobically incubated at 37°C for 24h (De Jong et al. 2003), respectively.

8.2.4 Screening test for spice effectiveness in broth media

Screening tests were carried out in nutrient broth (NB CM001, Oxoid Ltd., Basingstoke, Hampshire, UK) with some modification, NB was prepared by dissolving 13g of broth media plus 25 mg of phenol red (Sigma–Aldrich Company Ltd, UK) in one litre of distilled water then distributed in to 5 ml aliquots in bijoux tube (10ml bijoux tube, Oxide, UK) , using an Eppendorf dispenser (Eppendorf AG, 22331, Hamburg, Germany) and autoclaved at 121°C for 15 min. Phenol red was used as an indicator for growth/no growth of *B. cereus* and *C. perfringens*, when bacteria had grown, the media colour changed from red to orange-yellow, mainly due to the ability of these bacteria to produce acid. This method was used as a rapid test to determine the combined effect of time-temperature and spice addition on *B. cereus* and *C. perfringens* in broth media.

Test tubes were divided into three groups and each group was treated with different type of spice extracts (cinnamon, rosemary and sumac) at different concentrations ((High (0. 2), medium (0.125) and low (0.05) g/100ml of broth). Then, tubes were inoculated with 50 µl of 10^7 CFUml⁻¹ of *B. cereus* or *C. perfringens*, with a control positive (broth media plus the inoculum) and control negative (broth media only) were used for each treatment.

All treated tubes were covered and placed in a stainless steel wire basket and submerged in a temperature controlled water-bath (Grant instruments, Cambridge LTD, Barrington, England). These were previously stabilized at 55°C, and 60°C for *B. cereus* and *C. perfringens* vegetative cells. The sample temperatures in each water-bath were monitored continuously using thermocouple probes (K type), which were connected to a temperature logger (Comark Electronics, Ltd., Littlehampton, UK) and inserted into a tube with uninoculated broth. Once the broth reached the suitable temperature, tubes at 50°C were removed after 5 and 10 min for *B. cereus* and 10 and 20 min for *C. perfringens*, tubes at 60°C were removed at 1 and 2 min for *B. cereus* and 5 and 10 min for *C. perfringens*. The time required for the broth to reach the test temperature was monitored. Then the tubes were cooled down in iced water bath, and 0.1 ml of broth sample was plated (spread) directly onto nutrient agar, the tubes and plates were incubated aerobically at 30°C for 24-48 h for *B. cereus* and anaerobically at 37°C for 24-48 h for *C. perfringens*. After 24 h, tubes were examined for colour change and plates were examined for colony growth. This experiment was repeated three times with duplicate samples.

8.2.5 Sample preparation and inoculation

Lamb meat (3 kilos) from a local supplier was used to prepare doner kebab in the food and nutrition lab according to a simple traditional formula as detailed in Section 3.10.2. Kebab mix was divided in two batches, of 1500 g; one was treated with sumac extract at concentration of 0.2 (mg/100g of doner kebab); while the other was left without treatment as a control. For the sumac treatment, the corresponding concentration of sumac was added then mixed again for 5 min. This mixture was shaped and cooked as in Section 3.11, after cooking, control and sumac-treated slices were ground separately in a sterile food processor (Robot Coupe, R 402 v.v., France) followed by aseptically packaging into sterile polyethylene bags (Cryovac multiflex). Each bag contained 10 g of either control or sumac treated sample, was sealed using a Calor Bag Sealer (France), and kept frozen at -18°C until use. Before each experiment, the samples were thawed overnight at 4±1°C (Williams Refrigeration and Air-conditioning, installation by general refrigeration, Limited).

For the experiment, the bags were inoculated with 1ml, log 7 (10%) of the *B. cereus* or *C. perfringens* strain. Thereafter, the bags were manually mixed for 2 min to ensure even distribution of the inoculum in the doner kebab sample. then the bags were compressed into a thin layer (1–2 mm thick) by pressing them against a flat surface, excluding most of the gas, and then heat sealed by using a Plastic Film Sealer (FR-200, Sealing machine, WhenZhou LuCheng SongShan Welding Equipment's Factory, China).

8.2.6 Thermal inactivation and bacterial enumeration

For thermal inactivation studies, two replications were performed for the control and sumac treated RTE doner kebab samples. Bags at room temperature were placed in a stainless steel wire basket, and fully submerged in a temperature

controlled water-bath (Grant instruments, Cambridge LTD, Barrington, England). These were stabilized at 50, 55 and 60°C for *B. cereus* vegetative cells likewise; the water-baths were set at 55, 60 and 65°C for *C. perfringens* vegetative cells.

The sample temperature in each water-bath was monitored continuously using Thermocouples sensor (K type), which were connected to a temperature logger (Comark Electronics, Ltd., Littlehampton, UK) inserted into un-inoculated samples. Duplicate samples were taken at varying time intervals, from zero to 60 min, depending on treatment temperature and bacterial type. Subsequently, the samples were immediately placed in an ice-water-bath to cool.

All recovered samples were diluted with 90 ml MRD and directly homogenized (Stomacher 400, Colworth, London, UK) for 60 seconds, the resulting samples were serially diluted with MRD. An aliquot of 0.1 ml of the resultant solutions were spread-plated in duplicate onto *Bacillus cereus* agar (PEMBA) and incubated aerobically at 30°C for 24 h for *B. cereus* following the National Standard Method (Health Protection Agency 2004a).

For *C. perfringens*, 1 ml of sample solution was plated directly into an empty Petri dish then 20 mL of molten OPSP media was added, mixed well and left to set. After that (10 ml) of OPSP was used as an overlay and plates were incubated anaerobically at 37°C for 24 h (Health Protection Agency 2004b) before counting.

B. cereus appeared as large colonies (approximately 5 mm diameter), with a distinctive turquoise to peacock blue, and usually surrounded by a zone of egg yolk precipitate of the same colour, whereas, *Clostridium perfringens* were large black colonies (2-4 mm diameter) within the depth of the agar.

Experiment 2: Determination of time–temperature profile and calculation of process lethality in RTE doner kebab

8.2.7 Sample preparation and cooking protocol

The temperature profile for the lamb doner kebab roll was measured during cooking (grill) in order to assess the adequacy of the cooking process relative to recommendations of food authorities to minimise food-borne diseases due to pathogenic microorganisms. A frozen lamb doner kebab roll (5 kg) was purchased from K B S Factory as detailed in Chapter 3, Section 3.9.1 , and divided into 3 blocks (1.6 kg), (12 cm thick, 23 cm length and 44.5 cm diameter) prior to cooking, each frozen cylinder was thawed overnight at room temperature (about 22°C). At a trial day, doner kebab roll was inserted lengthwise on a doner kebab spit, and both ends of the cylinder were covered with melon to reduce heat traveling sideways into the sample area and to prevent sample dryness. The kebab roll was rotated every 30 second past an electric cooking element, this process continued until the whole roll was cooked. The temperature was measured by using six food-grade K-type thermocouple sensors, which were inserted, at two sites (top and middle) of doner kebab roll to its surface, middle and approximate geometric centre in such a way that the probes did not contact the spit of the grill. All thermocouples were connected to a temperature logger (Comark Electronics, Ltd., Littlehampton, UK), and temperatures recorded every 1min. The grill temperature was monitored using an infrared thermometer (Kane-May, KM 842, USA). This process continued until the temperature of the geometric centre of doner kebab reached 72°C for more than 2 min, as this condition is required for microbiological safety of processed meat products (McDonald et al. 2001) cited by (Goñi and Salvadori 2010).

8.2.8 Post cooking temperature distribution (temperature profile during cooling)

Post cooking temperature distribution was determined after cooking the sample. Doner kebab roll was removed from the grill and held on a tray to monitor the cooling profile at room temperature (22°C), where the temperature was recorded every 30 min until the geometric centre reached (22°C), this process took 5 hours. The cooling temperature was measured using three k type thermocouples connected to a temperature logger (Comark Electronics, Ltd., Littlehampton, UK).

8.2.9 Predicted the effect of both thickness and time on the cooking temperature of doner kebab roll

The time-temperature history of doner kebab roll at the surface, middle and approximate geometric centre were used to develop the response surface analysis model (RSA) using MINITAB (version 15). This model described the variation of the temperature as a function of the both time and sample thickness. The dependent variable was the temperature (C°), and the independent variables were the time (minute) and the thickness (cm) of the samples. A regression was performed using a second degree polynomial equation:

$$T(C^{\circ}) = b_0 + b_1 A + b_2 B + b_3 A^2 + b_4 B^2 + b_5 AB + \varepsilon \dots\dots\dots (8.1)$$

Where T: temperature (dependent variable modelled), and b_0 - b_5 are the coefficients to be evaluated, A: time, B: thickness, and ε : Random error. Only the terms that were statistically significant were considered for the RSA equation.

Additionally, heating rate was computed by using predicted temperature profile values of doner kebab roll at different thickness level and time following this equation:

$$\text{Heating rate}(C^{\circ}/\text{minute}) = \frac{T_1 - T_2}{dt} \dots \dots \dots (8.2)$$

Where, $T_1 - T_2$ is the differences between the predicted temperature at a given time and dt is the duration of time at a particular temperature (min).

8.2.10 Prediction of temperature profiles and computation of process lethality (F)

Time-temperature profiles demonstrate a curvilinear (polynomial Quadratic) behaviour where temperature increased during cooking of doner kebab roll until it reached 72°C. A temperature profile model was created by dividing the temperature profile into two areas as explained below:

8.2.10.1 Cooking doner kebab bulk (grill):

Temperature profiles for these regions were estimated as follows: Temperature profile during cooking and holding: The start temperature immediately was measured in duplicate pre-cooking samples using a hand held thermocouple probe and was 3.2°C. Temperature validation is always performed on the “cold spot” within a product (in our product the geometric centre of the doner kebab piece considered to have the lowest temperature), the lowest internal post cooking temperature for doner kebab roll was recorded as explained in Section 8.2.7. At the end of the heat hold time, the thermocouple always recorded temperatures of more than 72°C at all sites along the product centre, and that mainly due to high temperature in the middle and the surface of the product, which surrounded the centre of the product. Thus, it was supposed that, heat was still passing into the sample (heat transfers from a higher temperature area

to a lower area) this heat transfer is likely to appear in products with a large diameter (thick roll). Therefore, to get a predicted temperature at the end of grill heating, 1°C was subtracted from the temperatures measured at the end of the heat hold period. A curvilinear (polynomial) temperature profile was computed for the grill cooking cycle from the 3.2°C start temperature to this predicted temperature over 120 min. This model has the form of:

$$y = a + b_1x + b_2x^2 \dots\dots\dots (8.3)$$

Where y: is the predicted value of temperature (C°), a: is the intercept, b₁ and b₂: are the regression coefficients which is the change in temperature per time and x is the time (min).

8.2.10.2 Temperature profile during cooling:

Time-temperature profiles from the geometric centre of the doner kebab roll were computed to plot a cooling curve, samples (two doner kebab rolls) were cooled down and the data were recorded as described in Section 8.2.8.

The data obtained from the predicted cooling profile of doner kebab roll was used to predict the growth of *C. perfringens* by using the Pathogen Modelling Program PMP 70 (Cooling growth model), United States Department of Agriculture, Agriculture Research Service.

The process lethality (**F**) and log reduction of the process for both *B. cereus* and *C. perfringens* vegetative cells were computed using the predicted time temperature profiles and the *D* and *z* values for the doner kebab slices following these equations:

$$\text{Process lethality } f = \int_0^t 10^{\frac{T(t)-T_\theta}{z}} \times dt \text{ (Geankoplis 1993) } \dots\dots\dots (8.4)$$

Where, $T(t)$ is the predicted temperature ($^{\circ}\text{C}$) at the coldest point in the product at any time during the heat process, T_{θ} is the reference temperature and dt is the duration of time at a particular temperature (min).

$$\text{Log reduction of process} = \frac{F}{D} \dots\dots\dots (8.5)$$

Where, F is the process lethality of any treatment, D is the decimal reduction time of *B. cereus* or *C. perfringens* at a reference temperature.

Table 8.1: D and z values* for *B. cereus* and *C. perfringens* vegetative cells in RTE doner kebab Control (DK) and doner kebab with sumac extract (DKS) used to compute process lethality (**F**).

| Bacterial strain | Product | D_{θ} value (min)** | Z value ($^{\circ}\text{C}$) |
|-----------------------|---------|----------------------------|----------------------------------|
| <i>B. cereus</i> | DK | 2.9 | 9.11 |
| | DKS | 1.6 | 8.07 |
| <i>C. perfringens</i> | DK | 11.2 | 9.76 |
| | DKS | 9.9 | 9.3 |

*: D and z values taken from tables (8.4 and 8.5), ** D_{θ} : D -value at a reference temperature (60°C).

8.2.11 Statistical Analyses

Experiment 1

Statistical analyses and D-values and z-values Calculation

Three replicates of each experiment were done and performed in duplicate. D -values (time for 10-fold reduction in viable cells expressed in minutes), were established by plotting the thermal death curve (TDC) (the \log_{10} of the surviving cell ml^{-1} against time) for each individual temperature. To obtain the line of the best fit for each TDC, Linear regression analysis (Microsoft Excel 2007) was used; a regression equation of the type $y = a + bx$ was obtained, where a : intercept, b : the slope of the straight line. The average slope for a certain temperature treatment was used to compute D -values.

Each z-value (the thermal resistance of each bacterial group) was obtained by plotting the \log_{10} mean *D*-values against their corresponding temperatures and calculating the inverse negative of the slope of the thermal death curve (z-value = $-1/\text{slope}$) (Byrne et al. 2006).

The equivalent thermal destruction times were computed by using equation (8.6)

$$D_x = \text{Log}^{-1} (\text{Log } D_y - ((t_x - t_y)/z)) \dots \dots \dots (8.6)$$

One-way analysis of variance (ANOVA) and general linear model (GLM) were performed as detailed in Section 3.14 to verify whether the *D*-values for *B. cereus* and *C. perfringens* vegetative cell were different between control and sumac treated doner kebab samples at each heating temperature. And to check whether the z-values of *B. cereus* and *C. perfringens* vegetative cells were significantly different between control and sumac treated doner kebab; and finally to compare the z-value of *B. cereus* in the RTE doner kebab without treatment (control) and RTE doner kebab treated with sumac with those of *C. perfringens*.

Experiment 2

Replicate of this experiment were performed and the temperature was recorded by using six thermocouples. The effect of Time-temperature and heating rate on the surface, middle and centre of doner kebab roll during cooking was determined as in Section 3.14.

The regression coefficient was computed by using the polynomial quadratic model to quantify the effect of product thickness and time on the heating rate. To determine the process lethality and log reduction, AMI Foundation Facts and Figures was used (AMI Process Lethality Determination Spread sheet/ Microsoft Excel file).

8.3 Results

Experiment 1

8.3.1 Screening test for the antimicrobial effect of spice extracts combined with heat treatment in broth media against *B. cereus* and *C. perfringens*

The screening results for the combined effect of time-temperature and spice extract addition in broth media against vegetative cell of *B. cereus* and *C. perfringens* are represented in Tables 8.2 and 8.3.

The results mainly depended on the agar plating method, because change in colour was not valid for spice extract treated broth, the colour changes by spice extracts interfering with the indicator.

For *B. cereus* vegetative cells sumac and rosemary at high concentration (SH, RH) have a bactericidal effect when combined with the heat treatments (Table 8.2). While, for *C. perfringens* only SH has this bactericidal effect (Table 8.3). No effect was observed when cinnamon was used in this experiment against both bacteria. From this point, it was clear that a combined effect of sumac at high concentration with heating had a bactericidal effect versus both bacterial types at different time–temperatures. Therefore, sumac at high concentration was chosen to be used in a thermal inactivation study against spore-forming bacteria in ready to eat doner kebab.

Table 8.2: Effects of time-temperature and spice addition on the growth of *Bacillus cereus** in nutrient broth.

| Treatments | Temperature and Time | | | |
|------------|----------------------|--------|--------|-------|
| | 55°C | | 60°C | |
| | 5 min | 10 min | 1 min. | 2 min |
| Control | + | + | + | + |
| RH | + | - | - | - |
| RM | + | + | + | + |
| RL | + | + | + | + |
| CH | + | + | + | + |
| CM | + | + | + | + |
| CL | + | + | + | + |
| SH | - | - | - | - |
| SM | + | + | + | + |
| SL | + | + | + | + |

*: *Bacillus cereus* NCTMB11925; +: growth; -: no growth

RH: rosemary at 0.2%, RM: rosemary at 0.125%, RL: rosemary at 0.05%; CH: cinnamon at 0.2%, CM: cinnamon at 0.125%, CL: cinnamon at 0.05%; SH: sumac at 0.2%, SM: sumac at 0.125%, SL: sumac at 0.05%.

Table 8.3: Effects of time-temperature and spice addition on the growth of *Clostridium perfringens** in nutrient broth.

| Treatments | Temperature and Time | | | |
|------------|----------------------|--------|-------|--------|
| | 55°C | | 60°C | |
| | 10 min | 20 min | 5 min | 10 min |
| Control | + | + | + | + |
| RH | + | + | + | + |
| RM | + | + | + | + |
| RL | + | + | + | + |
| CH | + | + | + | + |
| CM | + | + | + | + |
| CL | + | + | + | + |
| SH | - | - | - | - |
| SM | + | + | + | + |
| SL | + | + | + | + |

*: *Clostridium perfringens* NACTC8239; for sample key see Table 8.2.

8.3.2 The decimal reduction time (*D-values*)

The decimal reduction time (*D-values*) of *B. cereus* and *C. perfringens* vegetative cells in RTE doner kebab were computed from the inverse negative slopes of the regressions at each temperature that are represented in Table 8.4 and Figures (8.1 and 8.2). The *D-values* in the control sample were higher than the corresponding *D-values* for sumac treated doner kebab sample for both *Bacillus cereus* and *C. perfringens* vegetative cell (Table 8.4).

The statistical analysis showed that the *D-values* are a function of heating temperature ($P < 0.0001$), pathogen types (*B. cereus*, *C. perfringens*) and treatments (control, sumac treated sample). Moreover, the statistics analysis confirmed that adding of 0.2g/100g sumac extract in RTE doner kebab reduced the heat resistance of *B. cereus* and *C. perfringens* at all time temperatures used, and the effect was quantified as significantly decreased *D-values* ($P < 0.05$).

Table 8.4: *D-value* means (min) of *B. cereus* and *C. perfringens* vegetative cells in RTE¹ doner kebab control (DK) and doner kebab with sumac extracts (DKS) at different temperatures.

| Bacterial groups | Treatments | Temperatures (°C) | R ² | <i>D-value</i> (min) |
|-----------------------|------------|-------------------|----------------|----------------------|
| <i>B. cereus</i> | DK | 50 | 0.979 | 36.7 ^a |
| | | 55 | 0.984 | 14.7 ^b |
| | | 60 | 0.978 | 2.9 ^c |
| | DKS | 50 | 0.986 | 28.5 ^d |
| | | 55 | 0.993 | 12.5 ^e |
| | | 60 | 0.998 | 1.6 ^f |
| <i>C. perfringens</i> | DK | 55 | 0.974 | 16.1 ^g |
| | | 60 | 0.989 | 11.2 ^h |
| | | 65 | 0.988 | 1.5 ⁱ |
| | DKS | 55 | 0.986 | 13.9 ^j |
| | | 60 | 0.989 | 9.9 ^k |
| | | 65 | 0.996 | 1.2 ^l |

¹: Ready to eat doner kebab; ^{a-l}: Means (n=3) within a column with different letters are significantly different ($P < 0.05$); R²: regression coefficient.

At each heating temperature, *B. cereus* and *C. perfringens* vegetative cells in RTE doner kebab followed first order kinetic, log-linear decline in surviving cells within time were observed. No evident lag periods or shoulders and tailing were manifest in any of the survivor curves for both bacteria (Figures 8.1 and 8.2). R^2 values of the microbial survival curves for *B. cereus* and *C. perfringens* vegetative cells, were greater than 0.97 (Table 8.4).

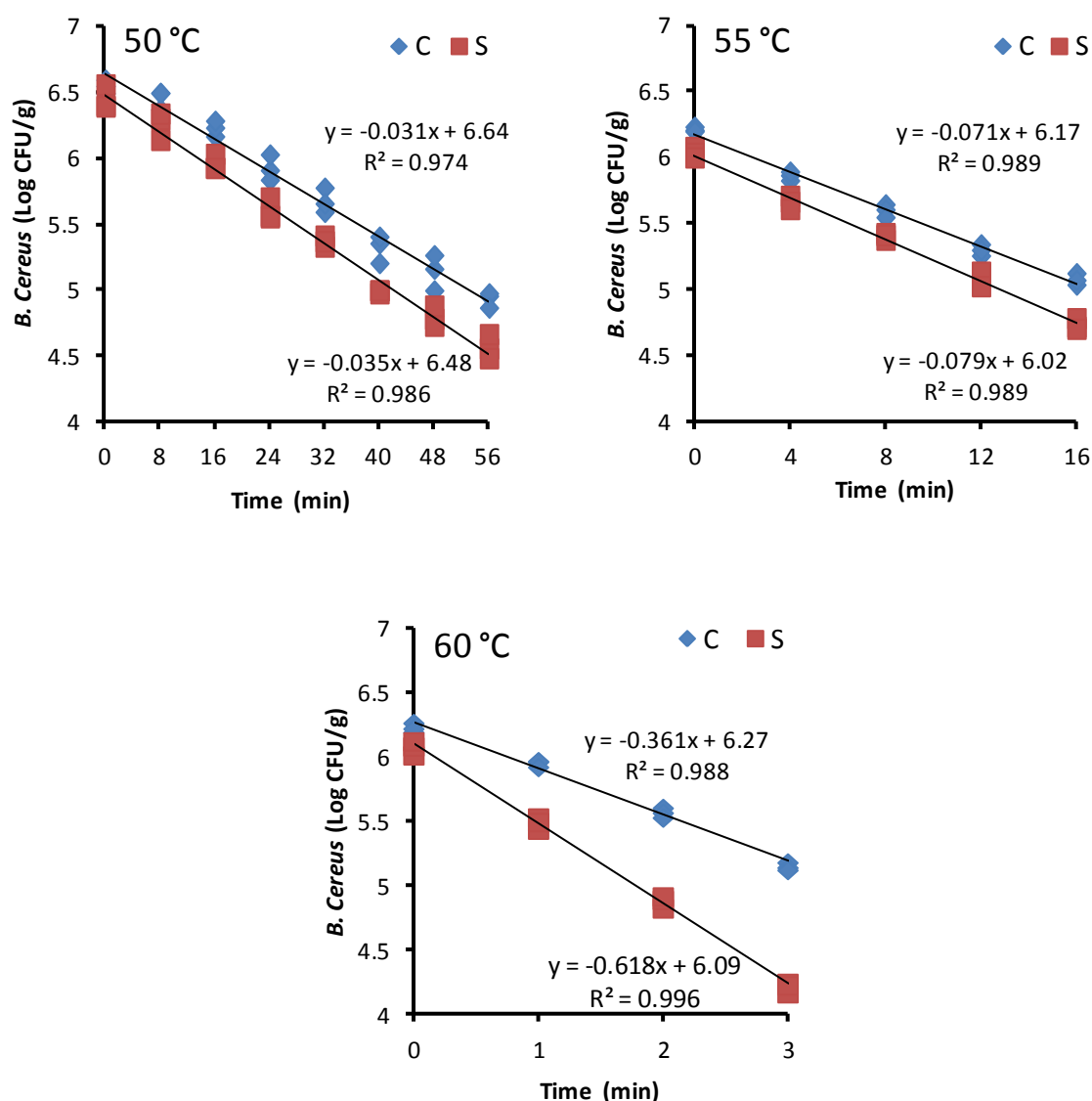


Figure 8.1: The microbial survival curves for *B. cereus* in RTE doner kebab sample (c: control, s: sumac) at different temperatures: 50°C, 55°C, and 60°C. Values are means for three readings.

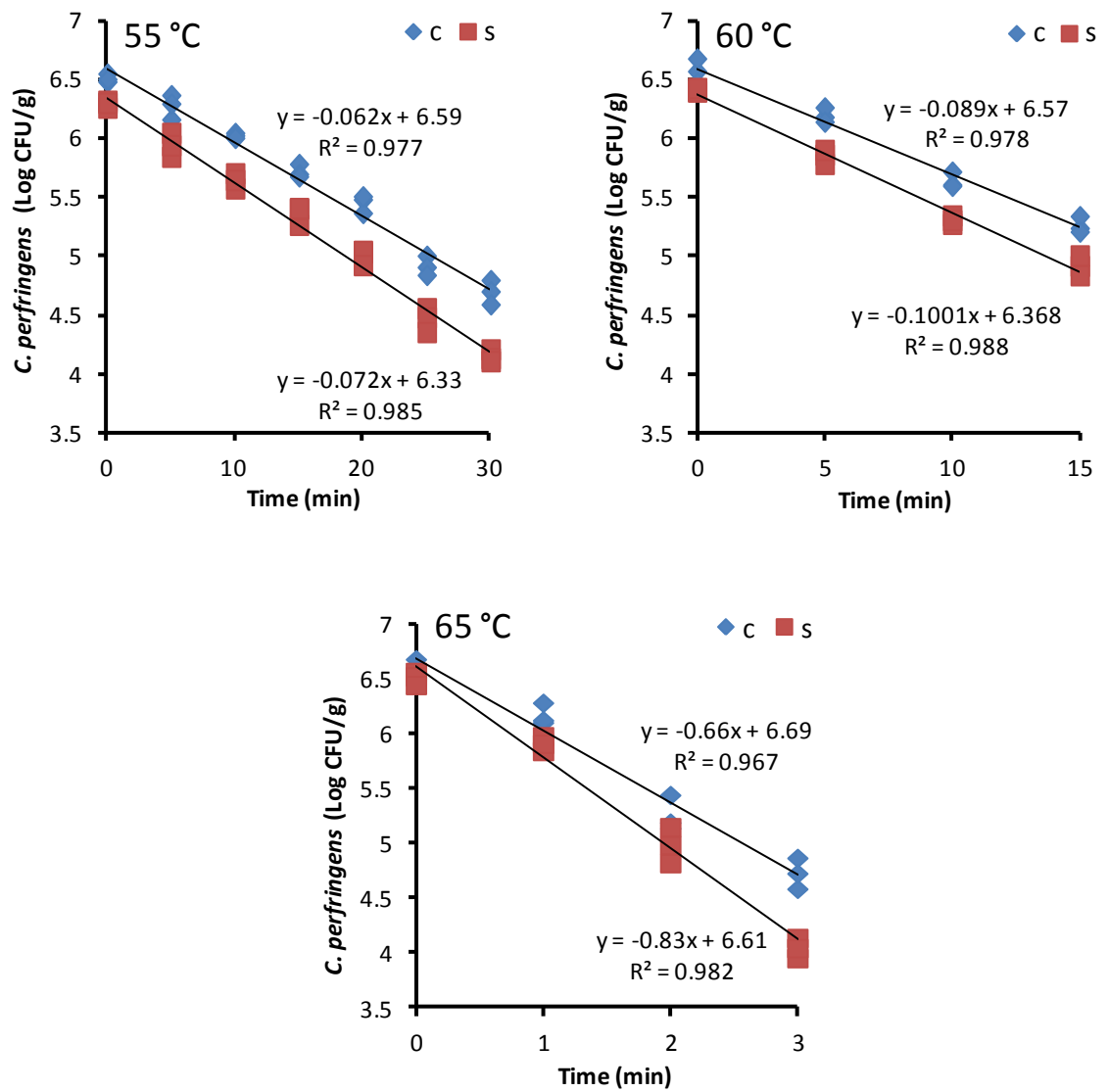


Figure 8.2: The microbial survival curves for *C. perfringens* in RTE doner kebab sample(c: control, s: sumac) at different temperatures: 55°C, 60°C, and 65°C. Values are means for three readings.

8.3.3 The thermal resistance (Z values) and predictive thermal destruction times for *B. cereus* and *C. perfringens* vegetative cells

The z-values were determined for both *B. cereus* and *C. perfringens* in RTE doner kebab by plotted linear regressions of $\log_{10} D\text{-value}$ (min) against heating temperatures as shown in Table 8.5. The z-values for *B. cereus* vegetative cells in control and sumac treated samples were 9.11 and 8.07°C respectively. Whereas, for *C. perfringens* vegetative cells z-values were 9.8 and 9.3°C correspondingly.

The statistical analysis shows that both the pathogen type (*B. cereus*, *C. perfringens*) and the treatment used (control, sumac treated sample) mainly affects the z-values. The results indicated that the z-values of control and sumac treated samples were significantly different for both *B. cereus* and *C. perfringens* ($P < 0.0001$, $P = 0.043$) respectively. In addition, z-values were significantly influenced by pathogen type ($P < 0.0001$).

Table 8.5: Z-value for *B. cereus* and *C. perfringens* vegetative cells for control and sumac-treated doner kebab.

| Bacterial groups | Treatments | R ² | z- Value (°C) |
|-----------------------|------------|----------------|-------------------|
| <i>B. cereus</i> | D K | 0.97 | 9.11 ^a |
| | D K S | 0.95 | 8.07 ^b |
| <i>C. perfringens</i> | D K | 0.96 | 9.76 ^c |
| | D K S | 0.95 | 9.30 ^d |

For sample key see Table 8.4, ^{a-d}: Means within a column with different letters are significantly different ($P < 0.05$).

Prediction the thermal destruction times for *B. cereus* and *C. perfringens* at different temperature in RTE doner kebab were obtained using equation (8.6) which is depicted in Figures 8.3 and 8.4.

It is obvious that *C. perfringens* vegetative cells were more heat resistant than those of *B. cereus* in lamb doner kebab for both control and sumac treated samples (Figures 8.3 and 8.4). The temperature equivalent computations predict that heating lamb doner kebab up to 70°C would considerably influenced the survival of *B. cereus* and *C. perfringens* vegetative cells. Therefore, to achieve six log reductions for *B. cereus*, the doner kebab samples should be heated up to 70°C for 1.43 and 0.56 min for both control and sumac treated sample respectively. whereas, for *C. perfringens*, a time temperature combination of 70°C for 2.8 and 2 min would be required to achieve 6 log₁₀ reductions in control and sumac treated sample correspondingly.

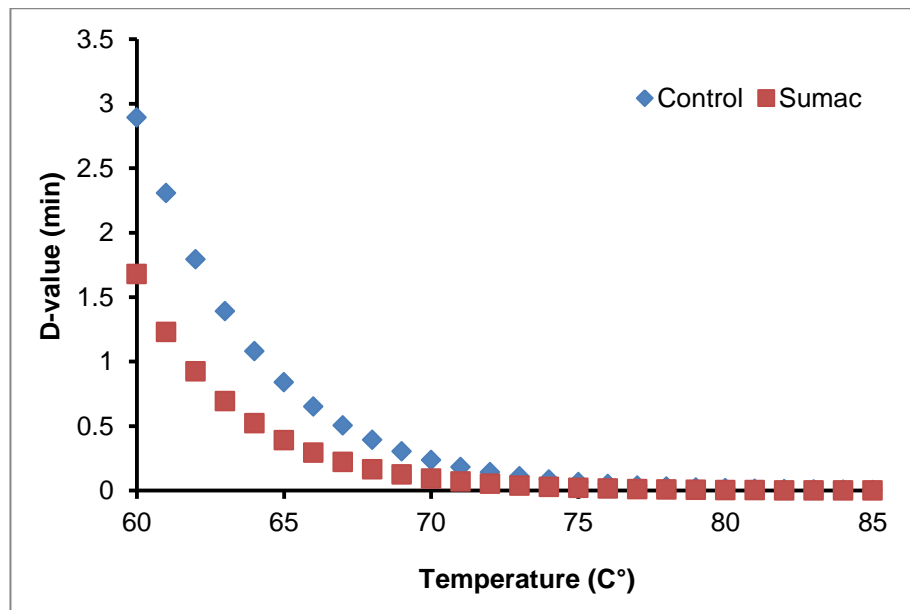


Figure 8.3: Time temperature required to achieve a 1 log₁₀ reduction of *B. cereus* vegetative cell in lamb doner kebab without spice (♦) and, doner kebab with sumac (■).

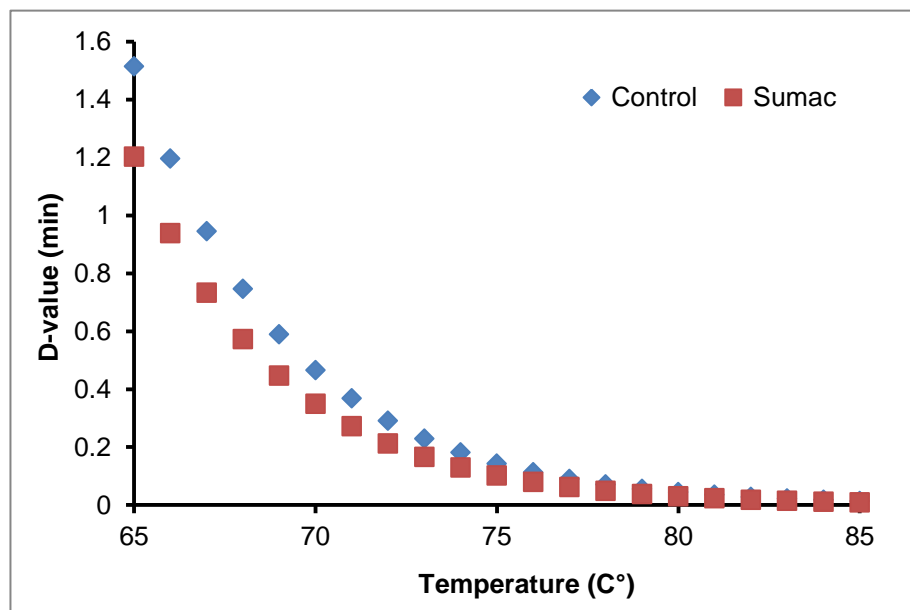


Figure 8.4: Time temperature required to achieve a 1 log₁₀ reduction of *C. perfringens* vegetative cell in lamb doner kebab without spice (♦) and, doner kebab with sumac (■).

Experiment 2

8.3.4 Time-temperature profile, end point temperature and heating rate

Time temperature profile of doner kebab roll is shown in Figure 8.5. The temperature was measured at the centre (core), middle part, and upper part (surface) during cooking. The surface temperature increased rapidly to the set point, and slightly fluctuated during cooking due to the cooking system, which was a one sided heating sources and rotating spit, thus the surface temperature heated up and down. The roll temperature increased gradually throughout the cooking period, with the surface reaching the highest temperatures, and the centre the lowest ($P < 0.001$), the difference in temperature from the surface to the centre of the doner kebab roll was $20.95 \pm 1.5^{\circ}\text{C}$ at the end of the experiment. The minimum, maximum and the temperature differences (ΔT) of doner kebab are illustrated in Table 8.6.

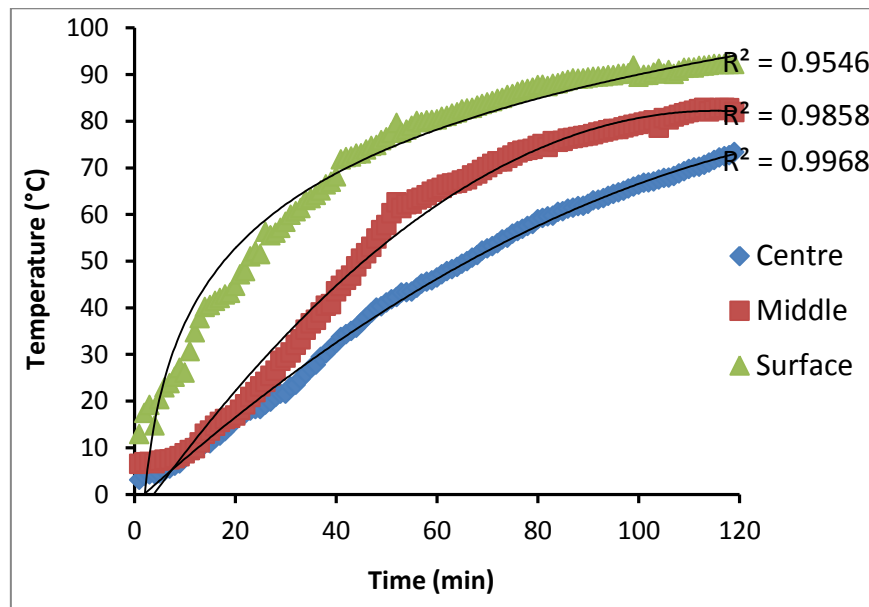


Figure 8.5: Time temperature profile for doner kebab. Data represent the mean of two replicate of doner kebab roll with six thermocouple readings.

Table 8.6: The minimum, maximum and temperature differences during cooking

| Temperature profile | Roll position | | |
|---------------------|-------------------|-------------------|--------------------|
| | Surface (S) | Middle (M) | Centre (C) |
| Minimum | 12.3 ^a | 05.2 ^b | 03.2 ^c |
| Maximum | 93.3 ^d | 83.2 ^e | 72.35 ^f |
| ΔC° | 81.0 | 78.0 | 69.15 |

^{a-f}: Means within a column or row with different letters indicate significant differences at ($P < 0.05$), ΔC° : temperature differences

The results obtained from the temperature profile of doner kebab roll showed that heating rate was time dependent and decreased as time progressed ($P < 0.05$). Whereas, no significant differences ($P > 0.05$) were observed among three levels thicknesses of doner kebab roll if we considering all cooking periods (0-120 minute) are considered. However, significant differences ($P < 0.05$) exist when the profile (120 min) was portioned into three stages (Table 8.7).

Table 8.7: Heating rate of doner kebab roll at different thickness levels and different time-temperature.

| Time (min) | Thickness (cm) | Heating rate* ($^{\circ}\text{C}/\text{min}$) | P-Value |
|------------|----------------|---|---------|
| 0-120 | 0 | 0.7 \pm 0.6 ^a | 0.787 |
| | 3 | 0.6 \pm 0.6 ^a | |
| | 6 | 0.6 \pm 0.4 ^a | |
| 0-40 | 0 | 1.1 \pm 0.8 ^a | 0.010 |
| | 3 | 0.8 \pm 0.7 ^{ab} | |
| | 6 | 0.6 \pm 0.6 ^b | |
| 40-80 | 0 | 0.6 \pm 0.3 ^a | 0.041 |
| | 3 | 0.8 \pm 0.5 ^{ab} | |
| | 6 | 0.8 \pm 0.3 ^b | |
| 80-120 | 0 | 0.3 \pm 0.2 ^a | 0.025 |
| | 3 | 0.3 \pm 0.3 ^{ab} | |
| | 6 | 0.5 \pm 0.4 ^b | |

*: heating rate presented as Mean \pm Standard error, ^{a-b}: mean within the row with different letter are significantly differences.

8.3.5 Predicted effect of product thickness and time on the cooking temperature of doner kebab roll

Response surface analysis model described the variation of the temperature (C°) of doner kebab roll as a function of the product thickness and time, using a polynomial quadratic form. The following equation was obtained

$$T(C^{\circ}) = 9.1 + (1.2Time) + (-4Thickness) + (-0.004Time^2) + (0.11Thickness^2) + (-0.007\{Time \times Thickness\})$$

The above equations estimated the predicted temperature profile under different thickness levels and different time's interval (Table 8.8).

The regression equation obtained from the response surface analysis model revealed that both time and thickness and their interaction had a significantly effect on the cooking temperature ($P < 0.001$).

Table 8.8: Response surface parameters of temperature as a function of both thickness and time.

| Parameters | Coefficients | Standard Error | P-value |
|-----------------------|--------------|----------------|---------|
| (Constant) | 9.125 | 0.64 | 0.000 |
| Time | 1.191 | 0.02 | 0.000 |
| Thickness | -3.950 | 0.26 | 0.000 |
| Time x Time | -0.004 | 0.00 | 0.000 |
| Thickness x Thickness | 0.111 | 0.038 | 0.004 |
| Time x Thickness | -0.007 | 0.002 | 0.000 |
| R ² | 0.986 | | |

R²: Coefficient of determination

The RSA model showed that doner kebab temperature increased progressively during the cooking time, and the lowest temperatures observed in the core of the product (higher thickness area) (Figure 8.6).

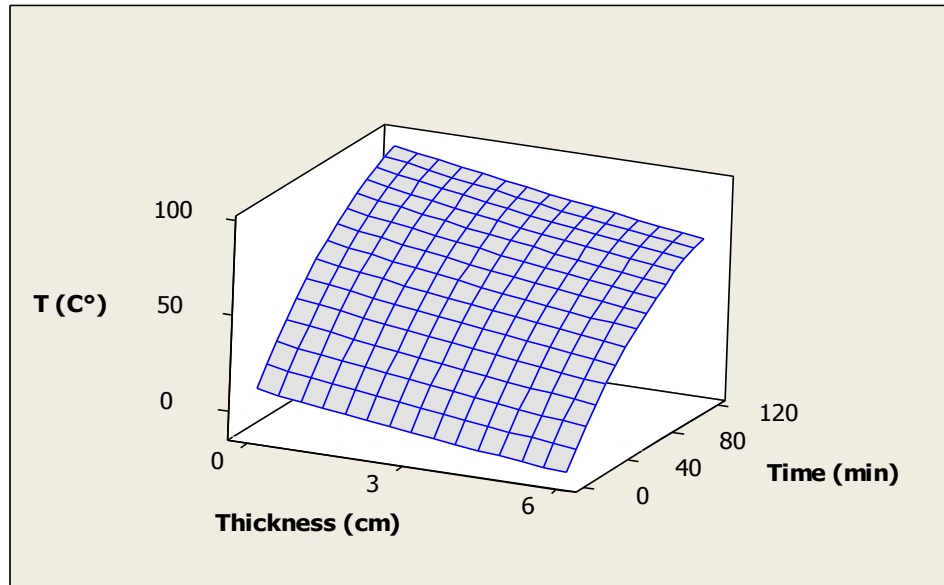


Figure 8.6: Predicted temperature profile as a function of combined effect of time and thickness and their interaction in doner kebab roll.

Predicted heating rate of three level thickness of doner kebab roll are presented in (Figure 8.7). The results indicated that heating rate was time dependent, which was decreased when the cooking time progressed. Moreover, no significant differences were observed among the samples thickness ($P > 0.05$).

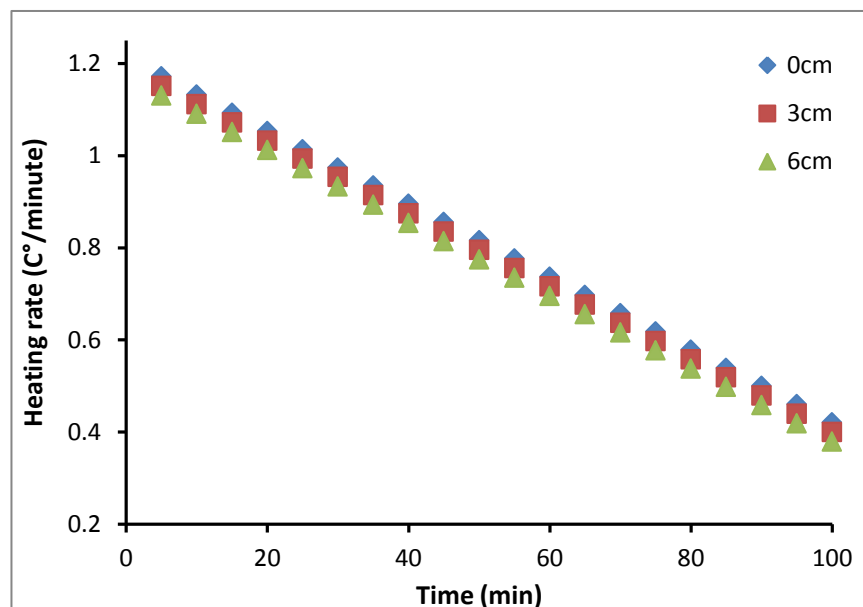


Figure 8.7: Predicted heating rate (C°/minute) as cooking time progress at three levels thickness 0, 3 and 6 (cm) of doner kebab roll.

8.3.6 Prediction of temperature profiles and computation of process lethality

8.3.6.1 Prediction of temperature profiles

A predicted time-temperature profile for both cooking and cooling of doner kebab roll demonstrated the characteristic curvilinear (polynomial) model, with $R^2 = 0.995$ (Figure 8.8). A typical time-temperature profile indicated that a total cooking time of 120 min was required to make sure that the doner kebab centre was heated above 72°C for more than 2 min. For a cooling profile at 22 °C for 5 hour the same scenario occurred with $R^2 = 0.998$ (Figure 8.8).

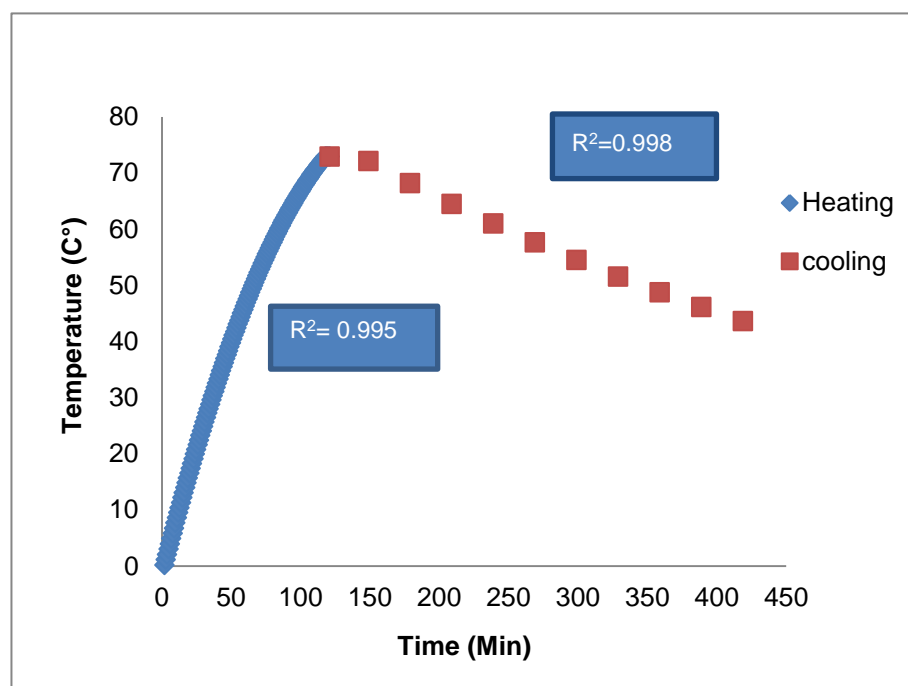


Figure 8.8: Predicted heating and cooling profile of doner kebab roll.

A pathogen Modelling Program / Cooling growth model was used to predict the growth of *C. perfringens* in doner kebab roll; the result indicated that cooling doner kebab samples at room temperature (22°C) for 5 hours, did not allow one log growth of *C. perfringens*. However, if the time is extending for 8 hours, one log growth will occur (Figure 8.9).

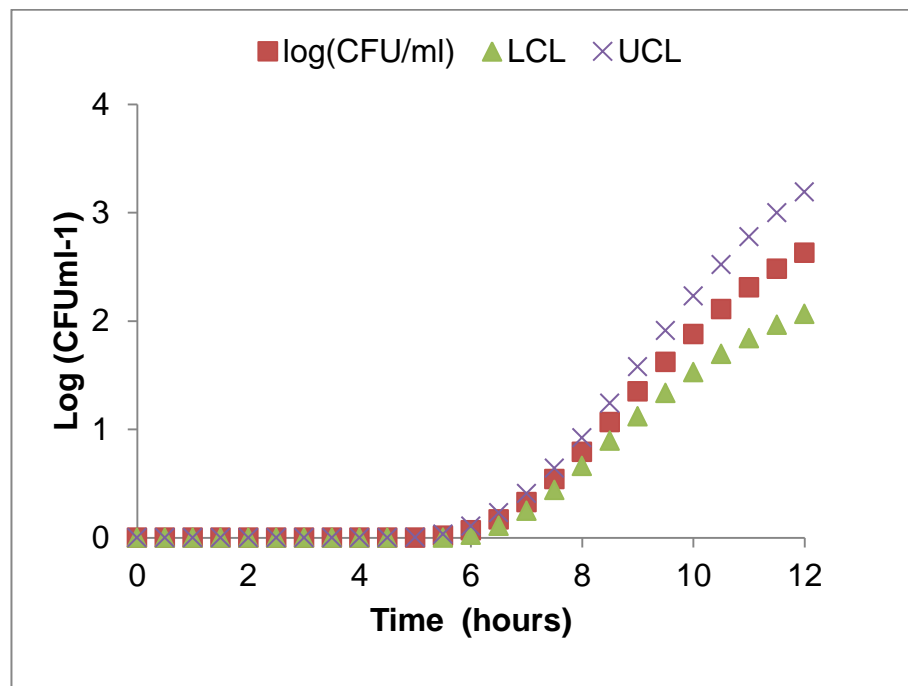


Figure 8.9: Predicted growth of *C. perfringens* at a cooling time of doner kebab roll at 22°C for 12hour. Log: predicted bacterial count; LCL: lower count limit; UCL: upper count limit.

8.3.6.2 Determination of process lethality and log reduction

The process lethality and log reduction of *B. cereus* and *C. perfringens* vegetative cells were computed using the temperature profile records at the centre of the doner kebab roll (Figure 8.10 and Table 8.9).

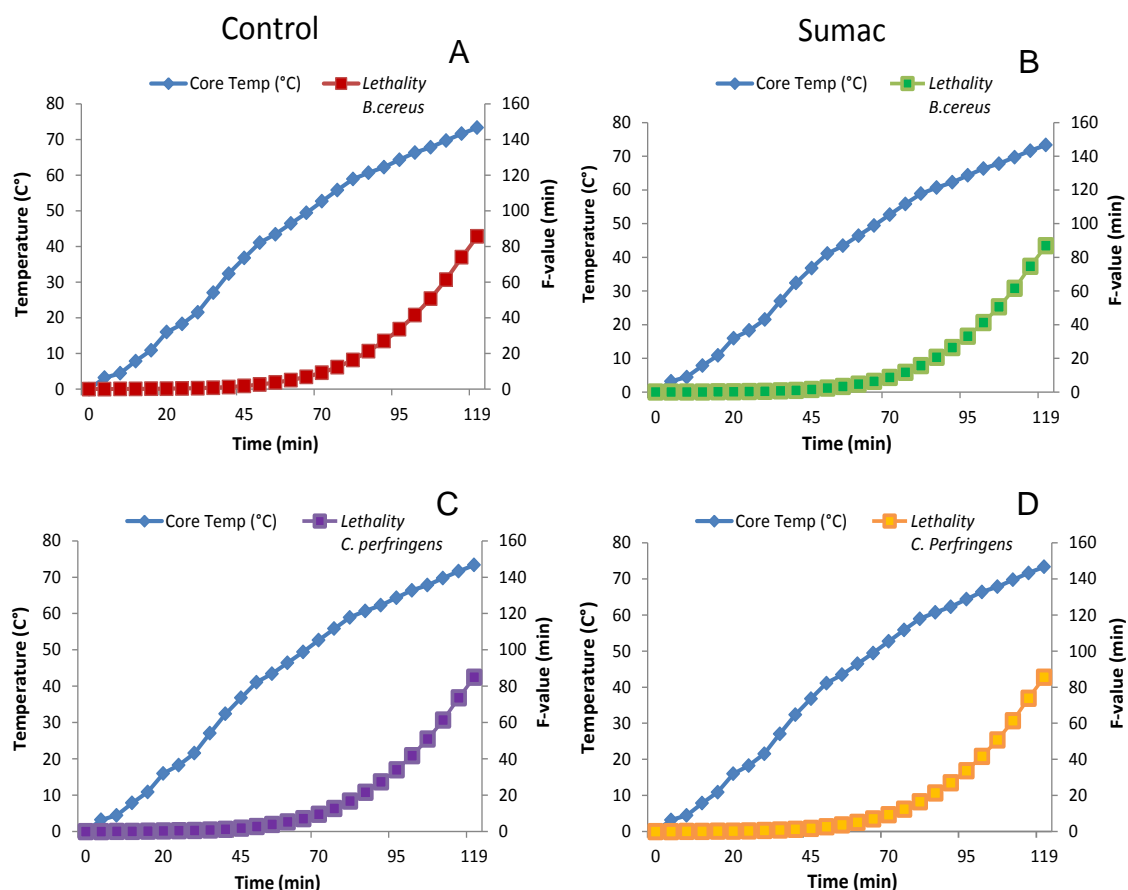


Figure 8.10: Centre temperature profile and process lethality of *B. cereus* and *C. perfringens* (calculated at a reference temperature of 60°C) in the cooked doner kebab roll for (A, C) control sample and (B, D) sumac treated sample.

The calculated process lethality for both *B. cereus* and *C. perfringens* in doner kebab samples were 86.8 min and 85.5 min in sumac and 85.7 and 85.1 min in control samples respectively. This lethality process achieved 54.3 log and 33 log reduction of *B. cereus* in sumac and control doner kebab sample respectively at 60 °C. Whereas, for *C. perfringens* at 60°C; 8.6 and 7.6 log reduction were achieved for sumac and control sample consequently.

Table 8.9: Time –temperature profile, process lethality (F value) and log reduction determination for *B. cereus* and *C. perfringens* in control(C) and sumac (S) treated doner kebab samples at 60°C.

| Time (min) | Core Temp (°C) | Process lethality (F value) | | | |
|---------------|-------------------|-----------------------------|--------------|--------------|--------------|
| | | <i>B.c</i> C | <i>B.c</i> S | <i>C.p</i> C | <i>C.p</i> S |
| 1 | 3.2 | 0 | 0 | 0 | 0 |
| 5 | 4.45 | 0.03 | 0.03 | 0.04 | 0.03 |
| 10 | 7.85 | 0.08 | 0.07 | 0.09 | 0.08 |
| 15 | 10.9 | 0.15 | 0.12 | 0.17 | 0.15 |
| 20 | 16.0 | 0.24 | 0.21 | 0.27 | 0.25 |
| 25 | 18.3 | 0.37 | 0.32 | 0.41 | 0.38 |
| 30 | 21.6 | 0.54 | 0.46 | 0.59 | 0.55 |
| 35 | 27.1 | 0.78 | 0.67 | 0.85 | 0.80 |
| 40 | 32.4 | 1.16 | 1.02 | 1.25 | 1.19 |
| 45 | 36.8 | 1.74 | 1.55 | 1.86 | 1.77 |
| 50 | 41.1 | 2.58 | 2.33 | 2.74 | 2.62 |
| 55 | 43.5 | 3.68 | 3.37 | 3.88 | 3.74 |
| 60 | 46.5 | 5.07 | 4.69 | 5.31 | 5.14 |
| 65 | 49.5 | 6.86 | 6.41 | 7.15 | 6.95 |
| 70 | 52.7 | 9.22 | 8.69 | 9.54 | 9.31 |
| 75 | 55.9 | 12.3 | 11.7♦ | 12.7 | 12.4 |
| 80 | 58.9 | 16.3 | 15.7 | 16.7 | 16.5 |
| 85 | 60.7 | 21.3♦ | 20.7 | 21.7 | 21.4 |
| 90 | 62.3 | 27.0 | 26.4 | 27.3 | 27.1 |
| 95 | 64.4 | 33.6 | 33.1 | 34.0 | 33.7 |
| 100 | 66.4 | 41.6 | 41.2 | 41.8 | 41.6 |
| 105 | 67.8 | 50.8 | 50.7 | 50.9 | 50.8 |
| 110 | 69.8 | 61.4 | 61.6 | 61.3 | 61.4♦ |
| 115 | 71.7 | 74.0 | 74.6 | 73.6♦ | 73.9 |
| 120 | 72.4 | 85.7 | 86.9 | 85.1 | 85.5 |

B.c.: *B. cereus*, *C.p.*: *C. perfringens*, C: control, S: sumac, ♦: six log reduction.

8.4 Discussion

Experiment 1

8.4.1 Screening test for the antimicrobial effect of spice extracts combined with heat treatment in broth media against *B. cereus* and *C. perfringens*

The use of phenol red in broth media as an indicator for growth /no growth of *B. cereus* and *C. perfringens* were valid for control samples only, since the colour changed from red to yellow after the incubation period, due to the ability of these bacteria to produce acid. Whilst, for broth treated with spice extracts this method was invalid, because when spice extracts were added to the broth, the colour changed immediately from red to orange or yellow depending on the spice type and concentration and regardless of the acidity. For this reason, the results relied on plate counts to determine the effectiveness of spice extract and time-temperature treatment in broth media.

At the different time-temperature combination used, sumac at high concentration exhibited considerable inhibitory effects (bactericidal effect) against *B. cereus* and *C. perfringens*. A similar effect was achieved with rosemary against *B. cereus* only when high concentrations were used. However, no bactericidal effect was noted for cinnamon at any concentration used (Tables 8.2 and 8.3).

The antimicrobial properties of spice extracts and their components have been previously assessed (Ceylan et al. 2004, Kong et al. 2007). The stronger antimicrobial activity of sumac extract is in agreement with literature reports by Al-Kutby et al.(2009), Nasar-Abbas and Halkman (2004), Nasar-Abbas et al. (2004) and Fazeli et al.(2007); and this activity could be caused by acidic (acetic acid, malic acid), or phenolic compounds present in sumac components (Zhao et al. 1997). Also, the antimicrobial effect of rosemary against spore-

forming bacteria in broth media is well documented (Valero and Salmerón 2003).

The present results indicated that the antimicrobial activity of sumac extract against *B. cereus* and *C. perfringens* was higher in broth media when compared with a meat product (doner kebab). As suggested by Larson et al. (1996) and Cutter (2000), meat and meat products are a complex growth environment, and the fat content in meat plays an important role in the antimicrobial activity of spice extracts, because it provides a protective coat around bacteria, thus protecting them from the antimicrobial agents. Additionally, Doyle and Mazzotta (2000) reported that food additives could be more effective in culture media than in foods as they might interact with protein and fat, which reduced their accessibility to bacteria.

8.4.2 The decimal reduction time (D-values)

The thermal death curve (TDC) for *B. cereus* and *C. perfringens* in RTE doner kebab followed a first order kinetic (log linear decline in the number of surviving cells with time), indicates that bacterial cells died at a constant rate, when the bacterial population was heated at a particular temperature (Figures 8.1 and 8.2).

The R^2 value for *B. cereus* and *C. perfringens* in this study were over 0.97. As reported by Byrne et al. (2006) and Juneja and Marmer (1998), R^2 value of more than 0.90, which achieves at least a 6 log reduction, might be considered linear. No lag periods or shoulders and tailing were observed in this study. As explained by Bolton et al. (2003), the heating media could confer a protective effect for bacterial cells. Thus, there was no clumping of bacterial cells (Stumbo

1973), no sub-populations with noticeably greater heat resistance (Murphy et al. 1999), nor formation for heat shock proteins (Pagan et al. 1997).

In the present study, *D-values* for *B. cereus* vegetative cells were found to be 36.7min D_{50} , 14.7 min D_{55} and 2.9 min D_{60} in control sample. Whereas, *D-values* for sumac treated sample ranged between (28.5 min. D_{50} - 1.6min D_{60}). There is a little information reported about the *D-values* for *B. cereus* vegetative cells. Doyle (2002), suggested that *B. cereus* vegetative cells are usually inactivated at temperatures higher than 60°C. Additionally, Byrne et al. (2006) found that the thermal destruction time (*D-values*) for *B. cereus* vegetative cells was 33.2 min. D_{50} , 6.4 min D_{55} and 1min. D_{60} in pork luncheon roll.

In general, the current results are consistent with those reported by Byrne et al. (2006) with some variation, the *D-values* for control doner kebab samples were found to be higher, in contrast the *D-values* for sumac treated doner kebab samples were found to be lower. These variations may be attributed to many factors such as: menstruum (percentage of protein, fat and carbohydrate in the product) (Jay 1986, Oteiza et al. 2003), type of meat, type of bacterial strain, previous exposure to stress (Pagan et al. 1997, Beales 2004), pH of the heating menstruum (Buchanan and Edelson 1999, Casadei et al. 2001), a_w of the heating menstruum (Gaillard et al. 1998, Mattick et al. 2000), and finally the preservative used.

The lower *D-values* reported by Byrne et al (2006) are perhaps due to the different type of meat product or different bacterial strain used. They used a luncheon product, formed from pork meat and normally treated with a curing agent that acts as a preservative. According to Doyle and Mazzotta (2000), food additives increase bacterial sensitivity to heat treatments. Furthermore, fat

percentage in luncheon meat is lower than in doner kebab. Thus, the *D-values* were expected to be lower than those obtained from lamb doner kebab (control sample). According to Smith et al. (2001), more heat-resistance of both *E. coli* O157:H7 and *Salmonella* Typhimurium DT104 were observed in beef containing 19% fat than in 4.8% fat beef. A protective effect of fat can be considered specially when the organism is exposed to dry heat (David and Norah 1991).

D-values in sumac-treated samples were found to be significantly different ($P < 0.05$) when compared with the corresponding *D-values* in control samples, with lower values obtained from sumac treated samples (Table 8.4). It is well documented that sumac has antimicrobial effects against both gram positive and negative bacteria. The antimicrobial activity of sumac is mainly due to an acid effect, sumac has a high percentage of citric and malic acid (5.02–14.24%) respectively, while an effect could also due to the phenolic compounds present (Nasar-Abbas and Halkman 2004, Fazeli et al. 2007). As suggested by David and Norah (1991), the heat resistance (*D-values*) of all microorganisms decrease as pH decreases (the decimal reduction time of most microorganisms is reduced 10 times when the pH decreases by 2 units).

For *C. perfringens* the thermal destruction time (*D-value*) ranged from 16.1 to 1.5 min in control samples, and from 13.9 to 1.3 min in sumac treated samples for D_{55} and D_{65} respectively. It is reasonable to compare the *D-values*, which were obtained in this study with those reported in previously published data. The *D-values* obtained in this study were generally in agreement with those reported by other authors. Byrne et al. (2006) reported similar *D-values* of 16.3 min D_{55} in pork luncheon meat, Juneja and Marmer (1998) found that the thermal destruction times for a *C. perfringens* vegetative cell cocktail were 17.5min for turkey and 21.6 min for beef at 55°C; Sarker et al.(2000) indicated

that *D-values* for *C. perfringens* vegetative cells in broth media ranged from (12.1-16.5 min D_{55}).

8.4.3 The thermal resistance (Z values) and predictive thermal destruction times for *B. cereus* and *C. perfringens* vegetative cells

The thermal resistance (z-values) for both *B. cereus* and *C. perfringens* vegetative cells in control and sumac treated samples are presented in Table 8.5. Adding sumac extract in lamb doner kebab samples reduced heat resistance for both bacterial groups, perhaps due to the antimicrobial effect of sumac as mentioned in Section 8.4.2. The z-values for *C. perfringens* were significantly higher than *B. cereus*.

In this study, the z-values for both bacteria were higher than those reported by Byrne et al. (2006), who reported that z-values for *B. cereus* and *C. perfringens* vegetative cells were 6.6 and 7.7°C respectively. Higher z-values were correlates to a slower temperature response to the \log_{10} D increase. In other words, a higher-z value means that a greater temperature rise is required to respond to a 90% increase in the decimal reduction time (D-value). Thereby, the pathogens are more tolerant to the changes in temperature (Murphy et al. 2004a).

The predicted time-temperature for both *B. cereus* and *C. perfringens* vegetative are presented in Figure 8.3 and 8.4. These are in agreement with the result obtained from Byrne et al.(2006) who reported that *B. cereus* was more sensitive to heat treatment than *C. perfringens* in luncheon meat.

Experiment 2

8.4.4 Time-temperature profile, end point temperature and heating rate

The time-temperature profile of doner kebab roll demonstrated that the surface of the roll was heated first with heat subsequently transferred to the colder interior (core). Accordingly, this can lead to overheating of the surface until the interior reaches a proper temperature (Figure 8.5 and Table 8.6). This is due to the heat conduction, when the heat transfers from the hottest area to the coldest area as a result of the present of a temperature gradient in the sample (Necati Özışık 1993).

The end point temperature (the cold point) was found to be located at the geometric centre of doner kebab sample and the temperature differential between the coldest and hottest points was 20.9°C at the end of the experiment (Table 8.6). That was perhaps due to several factors such as the type of cooking, size of the product piece and product composition (Ronald A and Stephanie 1994, Byrne et al. 2010).

Heating rate was significantly affected by product thickness when the profile (120 min) was partitioned into three stages. However, when the whole 120 min period was considered there was no significant difference. This could be attributed to the smoothing up of the difference during the course of the 120 min (Table 8.7)

The product near the surface (thinner part) heats at a fastest rate than in the centre (thicker part). This difference perhaps was resulting from a heat sink effect (Mangalassary et al. 2004). Throughout cooking, the heat from the grill cross the doner kebab surface and diffuses to the meat centre. The thicker the doner kebab sample, the better the diffusion of heat to the kebab centre, When the doner kebab roll was removed from the heat sources', the accumulated heat

then slowly diffuses back to the surface until the centre and surface reach the same temperature. In view of this phenomenon, thickness of doner kebab is a vital factor in achieving the required temperature. As reported by Mangalassary et al (2004), heating rate in Bologna product was fastest in the thinnest sample (4 mm) and slowest in the thickest sample (20mm) at different temperature. Murphy et al. (2002), indicated that the temperature gradient (heating rate) is mainly affected by the packaging thickness in fully cooked chicken breast meat. According to the time-temperature profile and end point temperature, it is clear that when the temperature reached the required degree on the surface, the middle and the centre of the product are still in an at risk temperature zone. An additional problem occurs due to the dripping of the uncooked fluid from the inside of the product to the outside and that could be a potential hazard (especially if the raw product was contaminated) thus, yielding the final product unsafe.

8.4.5 Prediction of temperature profiles and computation of process lethality

The statistical indices represented by R^2 obtained from the secondary model were illustrated in Table 8.8. The R^2 values were relatively high, indicating adequacy of the data to fit the model, the higher value of R^2 ($0 < R^2 < 1$) the better is the prediction by the model (Grau and Vanderlinde 1993).

The RSA model showed that both time, thickness and their interaction had a significantly effect on the cooking temperature of doner kebab roll (Figure 8.6) and the heating rate is time dependent, decreasing as the time progresses (Figure 8.7). The most probable explanation for this is when the cooking time progresses the temperature differences in the sample decreases.

8.4.6 Predicted the effect of product thickness and time on the cooking temperature of doner kebab roll

8.4.6.1 Prediction of temperature profiles

The predicted temperature profile for doner kebab roll showed curvilinear (polynomial) characteristics for heating and cooling profiles, with higher R^2 values (Figure 8.8), this may be due to the fact that for this type of product the heat transfer happened mainly by conduction (Food Safety and Inspection Service and United States Department of Agriculture 2003). As suggested by Zhang et al. (2004) a characteristic curvilinear time-temperature profile was a typical in product where heat transfer takes place primarily by conduction.

Exposures to heat treatment during cooking and anaerobic conditions may enhance the activation of *C. perfringens* spores. In general, heat-resistant spores of *C. perfringens* can be activated by heating at 65–100°C for 10-20 min (Walker 1975, Doyle 1989). Although heat treatment can activate spores, at the same time, spore damage can occur, but germination will happen if the damage

is repaired. To repair such damage, rich media such as meat is required (Barach et al. 1975, Labbe and Chang 1995).

For *B. cereus* spore germination, aerobic conditions are required and not all strains require heat activation to germinate the spores. It is well documented that *B. cereus* germination happens at a wide range of temperatures and the optimal temperature for germination is 30°C (Doyle 1989). Therefore, long cooking time for doner kebab that will heat up and down rather slowly could enhanced the germination and increase the resistance of spore forming bacteria specially *C. perfringens* (Smith et al. 1981, Roberts et al. 1995).

When doner kebab rolls were left at room temperature, product temperature decreased from 72 - 22°C during 5 hours (Figure 8.9), it was expected that the cooling process would not be fast enough to prevent multiplication of *C. perfringens* and other types of spore forming bacteria.

As suggested by Kalinowski et al. (2003), during the cooling process of meat products, *C. perfringens* spores will germinate and grow due to the absence of oxygen, and maximum growth occurs at temperatures from 54.5 -15.5°C. Thus, in meat products (especially red meat) a primary pathogen of concern during cooling is *C. perfringens* (Roberts et al. 1995, Doyle 2002, Fazil et al. 2002).

According to the U.S. Centres for Disease Control and Prevention (CDC) (1996), a viable count of 10^5 or greater of *C. perfringens*/gram have been suggested as one of the criteria for implicating *C. perfringens* as the causative agent of food-borne illness in RTE product (CDC, 1996).

Food Safety and Inspection Service and United States Department of Agriculture (Food Safety and Inspection Service 1999b), issued a performance standard of the ready-to-eat meat products to inhibit the germination and multiplication of spore forming bacteria and permit no more than a one \log_{10}

multiplication of *C. perfringens*. Thus, limiting the fast growth bacteria such as *C. perfringens* to a 1 log₁₀ multiplication would be successfully inhibit the growth of other slower growing spore-forming bacteria, such as *B. cereus* and *C. botulinum*.

Additionally, Food Safety and Inspection Service and United States Department of Agriculture (1999b), issued a performance standard: Guidelines for cooling heat-treated Meat and Poultry Products (Appendix B). This guideline was the stabilization performance standard for safe cooling of these products which focus on the germination and outgrowth of spores during cooling. The compliance guidelines suggested that processed meat and poultry products for stabilization state must be cooled down from 54.58 to 26.68°C within 1.5 h and from 26.6 to 4.48°C within 5 h.

On the other hand, the Campden and Chorleywood Food Research Association (UK) recommend that uncured meat products must be cool down from 50 to 12°C within 6 hours and from 12 to 5°C within 1 hour if the level of contamination is low. However, if the product is highly contaminated, these recommended cooling times may be not enough to ensure safety (Gaze et al. 1998).

C. perfringens spore and other spore-forming bacteria can survive cooking and, actually, thrive in the warm product. (Doyle 2002). Thus, a rapidly cooling regime must be applied after cooking.

As a result of the time limitation of the current study, a Pathogen Modelling Program/Cooling Growth Model was used to predict the growth of *C. perfringens* in doner kebab roll during cooling time. The results indicated that cooling doner kebab at room temperature (22°C) for 5 hours, did not allow one

log growth of *C. perfringens*. However, if the time is extended to 8 hours one log growth will occur (USDA standard) Figure 8.9.

Growth prediction of *C. perfringens* where mainly depend on the result obtained from Juneja et al.(1999) in beef broth, because Juneja et al. developed a model to predict the growth of *C. preferences* for specified temperatures throughout the entire cooling temperature range of the cooked foods and he used equations for *C. perfringens* which is appropriate for foods of neutral pH, high water activity and absence of other anti-microbial agent. Previous results indicated that doner kebab product has a similar characteristic feature (chapter 5) corroborating other authors (Kayisoglu et al. 2003, Gonulalan et al. 2004).

Although, Pathogen Modelling Program is a widely used model to predict the effect of multiple variables on the growth, survival and inactivation of food-borne pathogens, most models are based on extensive experimental data of microbial behaviour in liquid microbiological media. Thus, further research is needed (challenge test) to study the kinetics growth of *C. perfringens* in doner kebab roll as a function of the holding and cooling time.

8.4.6.2 The process lethality and the log reduction

Process lethality was integrated from thermal history; heating rate in a process affected the thermal history and consequently affected the process lethality. The process lethality and the log reduction of control and sumac treated doner kebab are illustrated in Figure 8.10 and Table 8.9.

It is clear that the product type (sumac and control doner kebab sample) did not affect the process lethality and that was expected because the same heating schedule was used, but the significant effect occurred on the log reduction of *B. cereus* and *C. perfringens* for control and sumac treated doner kebab samples. There was a high log reduction in sumac treated sample for both bacterial groups, and that was perhaps due to the effect of sumac extract on the *D* and *Z* values (low *D* and *Z* values were obtained from sumac treated doner kebab sample) for both bacteria. Moreover, a lower log reduction was obtained from *C. perfringens* than *B. cereus*, which was definitely due to high thermal resistant of *C. perfringens* (Heredia et al. 1997, Byrne et al. 2006).

In the current product, and according to the process lethality, a 6.5 log reduction in *B. cereus* at 60°C could be achieved at 11.7 and 21.3 min in sumac and control sample respectively, while for *C. perfringens* cooking time of 61.3 and 73.6 min were required to achieved 6.5 log reductions for sumac and control sample correspondingly.

The higher process lethality observed in this study could be due to the heat transfer in this type of product, which is rather slow. Murphy et al. (2004b) reported that slower heating rate gave higher process lethality during cooking and fully cooked meat product (frankfurter sausages).

Therefore, during cooking the time -temperature history of the products, is crucial in computing the process lethality for the concerned pathogen. In

present study, the calculated process lethality of control and sumac treated sample for *B. cereus* was greater than the processing time that was required for achieving a 6D reduction (17.4 and, 9.6 min) for control and sumac treated sample respectively at 60°C. Whereas, for *C. perfringens* to achieve 6D reduction at 60°C, 59.4 and, 67.2 min were be required, computed from equation (8.6). This is not too dissimilar from the time we obtained from the process lethality. However, it should be highlighted that the process lethality is a mathematically computed value therefore; validation of the process was necessarily to reflect the real kills of bacteria.

8.5 Conclusions

The recent growth of the market for RTE doner kebab has led to serious concern over food-borne pathogens; particularly spore forming bacteria. Emphasis has been placed on both thermal processing and spice addition to eliminating food-borne pathogens in meat product. The results confirmed that application of sumac could improve the safety of RTE doner kebab in regards to spore forming bacteria as the risk related to survival is reduced. Thus, reformulating the product could be useful to assure the thermal inactivation of spore forming bacteria.

Determination of the temperature profile of RTE doner kebab roll helped to measure the integrated time-temperature processes. This can be used to optimize the cooking process. Therefore, we can get the desired performance standard with less time.

The model obtained from *C. perfringens* during the heating and cooling profile in this study need farther investigation. Such investigation is by predicting the safe depth of doner kebab slice after shaving and validation the data before it is used in commercial circumstances or regulatory purposes.

Chapter 9

9 Predictive Model of *Listeria monocytogenes* and *Salmonella* Typhimurium Growth Rate under Different Temperatures, pH, NaCl, and Spice Extracts

9.1 Introduction

The increasing number and severity of food-borne outbreak's world-wide has increased consumer awareness about the safety of meat and meat products (Adrian 1996). Consumer demand for fresh or minimally processed healthy food with natural additives has generated a call for quick and accurate methods to guarantee food safety.

Several mathematical models (microbial growth models) have been developed and used to predict the likelihood and extent in the growth of food-borne pathogens and spoilage bacteria under specific environmental conditions such as temperature, pH, sodium chloride, water activity, and preservatives (Farber et al. 1996, George et al. 1996, Blackburn et al. 1997, McClure et al. 1997, Francois et al. 2005, Carrasco et al. 2006, Pin et al. 2011). These models allow the food microbiologist not only to get an accurate prediction to obtain safe and good-quality meat products (Juneja et al. 2009, Couvert et al. 2010), but also help to determine the critical parts of the production and distribution process (Koutsoumanis et al. 2005, Nychas et al. 2008, Rhoades et al. 2009).

Furthermore, investigators need adequate models to describe the growth curves and to reduce measured data to a limited number of interesting parameters (Baranyi and Roberts 1994).

As a result of the time-consuming nature of traditional plate-count techniques, the use of faster and more convenient data collection methods are required (Benoit and Donahue 2003). One of the alternative methods is based on Absorbance measurements (Dalgaard and Koutsoumanis 2001, Biesta-Peters et al. 2011) which has the advantage of being-rapid, inexpensive, non-destructive and relatively easy to automate. Additionally, predictive models obtained from absorbance measurements data are reliable, generally validate well against models, and provide a favourable estimation of microbial response (Gill and Holley 2003, Banja 2010). Therefore, some researchers have built growth models of pathogenic bacteria based on absorbance data (Carrasco et al. 2006, Valero et al. 2006).

The aims of this study were to investigate the combined effect of temperature, pH, sodium chloride and spice extract addition on the growth kinetics of *L. monocytogenes* NCTC7973 and *S. Typhimurium* DT104 and use data to build a growth model

9.2 Materials and methods

9.2.1 Bacterial strains

Culture collection strains of *Listeria monocytogenes* NCTC7973 (type strain) was purchased from the national collection of type cultures (A Health Protection Agency Culture Collection, Centre for infections, London, UK). *Salmonella* Typhimurium DT104 (human isolate), was obtained from the University of Plymouth.

The culture collection inocula were prepared as follows: each bacterial strain of *L. monocytogenes* and *S. Typhimurium* was maintained on brain heart infusion agar (Oxoid Ltd., Basingtoke, Hampshire, UK) at $4\pm1^{\circ}\text{C}$. A loop-full of a fresh subculture of *L. monocytogenes* and *S. Typhimurium* were used for inoculation in fresh BHI broth and incubated aerobically over night at 37°C (when the early stationary phase was reached). The number of microorganisms in the broth media was determined by using a McFarland standard's opacity tube (Harrigan 1998). These bacteria were selected according to the potential hazard in ready to eat meat products (Gilbert et al. 2000) and also they account for almost 60% of the burden of food-borne disease in UK (Operational Research Unit and Analysis and Research Division 2011).

9.2.2 Experimental design

A full factorial design incorporating the following conditions: three temperatures, three NaCl concentrations, three pH levels and three types of spice extracts at three concentrations level high (H), medium (M), and low (L) were used to investigate the growth kinetics of *L. monocytogenes* and *S. Typhimurium* under different combination conditions. The conditions for the tests are shown in Table 9.1.

Table 9.1: Conditions tested for absorbance measurements during the experiment.

| Time (hours) | Temperature (°C) | % NaCl | pH levels | Spice extracts | |
|-----------------|---------------------|--------|--------------|----------------|--------------------------------|
| | | | | Types | Levels (%) |
| 500 | 5 | 0.5%. | 5 | Cinnamon | L= 0.05 M= 0.125 H= 0.2 |
| 250 | 15 | 1.5% | 6 | Rosemary | L= 0.05 M= 0.125 H= 0.2 |
| 24 | 30 | 5.5% | 7 | Sumac | L = 0.05 M= 0.125 H= 0.2 |

L: low concentration, M: medium concentration, H: high concentration.

9.2.3 Preparation and inoculation of culture media

Brain heart infusion broth (Oxoid Ltd., Basingtoke, Hampshire, UK) was used as a basal medium for the growth and maintenance of *L. monocytogenes* and *S. Typhimurium*. The basal media was prepared as detailed in Chapter 3, Section 3.7 and the pH was adjusted to five, six, or seven by using 1M NaOH or 1M HCl. NaCl was then added to the basal media to provide three concentrations of NaCl for each pH conditions. Afterward, spice extracts were added for all pH conditions and all NaCl concentrations. This pH adjusted media was sterilised by autoclaving at 121°C for 15 minutes then stored at 4±1°C (Shin-Young, Kyo-Young and Sang-Do 2007).

9.2.4 Growth temperature and growth rate measurement

Growth rates for *L. monocytogenes* were determined at 4, 15 and 30°C, whereas, for *S. Typhimurium* growth rates were determined at 15 and 30°C only. Both total viable counts and turbidity measurements were carried out as mentioned below:

9.2.5 Viable count measurements

Total viable count (TVC) measurement was performed every two hours for *L. monocytogenes* and *S. Typhimurium* at 30°C, four hours at 15°C and 10 hours at 4°C incubation temperature for 24, 48 and 110 hours respectively. At each corresponding time, 0.1 ml of the aliquots of serial dilution was plated on BHI agar, duplicate for each dilution. Then the plates were incubated at 30°C for 48 h. Plates with colony-forming units ranged from 30 to 300 were counted and log-transformed to homogenise variances.

9.2.6 Turbidity measurements

A micro-plate automated reader (TECAN, Infinite 200, Reading, UK) was used to obtain the absorbance measurement for both *L. monocytogenes* and *S. Typhimurium* using flat bottom transparent 48 well plates (Corning, fisher Scientific). Microplate wells were topped up with 850 μl of each medium combination of NaCl, spices and pH then 50 μl of inoculum containing 10^5CFU ml^{-1} of either *L. monocytogenes* or *S. Typhimurium* was added. A kinetic cycle of 24h with a shaking duration 5s performed before each one-hour measurement. Measurements were programmed for each growth experiment (A total of 270 and 180 different conditions for *L. monocytogenes* and *S. Typhimurium* respectively were obtained for the model). The absorbance measurements were done in triplicate at a wavelength of 690nm and bandwidth of 19 nm (Banja 2010).

9.2.7 Primary model

9.2.7.1 Baranyi and Roberts model

Growth curve against time obtained from viable count measurements TVC and turbidity measurement for *L. monocytogenes* and *S. Typhimurium* were fitted to Baranyi and Roberts (1994) model by using MicroFit version 1 (Institute of Food Research, Norwich, UK), conditions in which growth was not observed, were not included.

This model predicted the kinetic parameters for bacterial growth such as: initial bacterial density ($N_0 \text{ CFU ml}^{-1}$), final bacterial density ($N_{\text{max}} \text{ CFU ml}^{-1}$), and maximum specific growth rate ($\mu_{\text{max}} \text{ h}^{-1}$), lag time ($t\text{-lag h}$), and doubling time ($t\text{-d h}$).

In addition, this model calculated an estimate of the error associated with the parameter value (data related to the goodness of the fit of the model) e.g.: The residual sum of square (RSS) and the Root Mean Square Error (RMS). The former considered as an indicator of the goodness of the fit, and it is used in the computation of the error on the model parameter, whereas the later was providing a more intuitive measure of how well, the model fits the data.

Moreover, this model performs a statistical comparison between two data sets by comparing two models. A statistics test to establish the significance of the contribution of each model parameter test for the two data sets, were performed with MicroFit software. The first model, named the 'Separated' model, has seven parameters, i.e. values of N_0 , N_{\max} , μ_{\max} , $(t-d)$, and $t\text{-lag}$ for each of the two data sets. Whereas, the second model which is called the 'Unified' model, has only five parameters. The statistical test determines whether if there is a significant improvement in the fit to the data sets by using the 'Separated Model' rather than the Unified Model (Baranyi and Roberts 1994).

9.2.7.2 Gompertz model

Growth curves obtained from viable count measurements TVC for both *L. monocytogenes* and *S. Typhimurium* were fitted to a Gompertz equation using a nonlinear regression model (Sigma Plot Version 11). The US Department of Agriculture (USDA) has developed this model to predict the microbial growth in a food environment containing several controlling parameters (Ray 2005). The Gompertz model produced an output including lag time, maximum growth rate and maximum microbial load directly from nonlinear regression of the growth value against time.

The Gompertz sigmoidal equation is:

$$N = A + C \exp(-\exp[-B(t - M)]) \dots\dots\dots (9.1)$$

Where N is \log_{10} (CFUml⁻¹) at the time t , A the initial \log_{10} CFUml⁻¹, C the \log_{10} CFU difference between time t and initially, \exp is exponential, B the relative growth rate at M , and M the time at which growth rate is maximum.

9.2.8 Secondary model

The kinetic parameter (maximum growth rate) obtained from the Baranyi and Roberts model for both bacterial groups (Turbidity measurement) were used to develop the response surface analysis model (RSA) using Minitab (version 15). This model described the variation of the growth rate as a function of the growth conditions. As previous studies indicated that maximum growth rate can be predicted with a high degree of accuracy, because it is directly response to the current growth environment (Rosso et al. 1995, Baranyi and Pin 2001, Marina et al. 2010). Whereas , lag phase predictions as a function of the current growth conditions are generally highly inaccurate, since the lag phase duration is influenced by many factor including, the current and the previous growth conditions, stresses, and cell physiological states (Augustin et al. 2000, Mellefont et al. 2003, Pin and Baranyi 2008 , Marina et al. 2010).

The dependent variable was the maximum growth rate ($\mu_{\max} \text{ h}^{-1}$), and the independent variables were temperature, pH, NaCl concentration and spice extracts types with different levels. A regression was performed using a second-degree polynomial equation:

$$\begin{aligned} \mu_{\max} = & b_0 + b_1A + b_2B + b_3C + b_4D + b_5A^2 + b_6B^2 + b_7C^2 + b_8D^2 + b_9AB \\ & + b_{10}AC + b_{11}AD + b_{12}BC + b_{13}BD + b_{14}CD + \varepsilon \dots \dots \dots (9.2) \end{aligned}$$

Where μ_{\max} : maximum growth rate (dependent variable modelled), and b_0 - b_{14} are the following coefficients, A: incubation temperature, B: pH, C: NaCl concentration, D: spice extracts types and ε : random error. Only the terms that were statistically significant were considered for the RSA equation.

9.2.9 Evaluation of model performance:

The performance of the predictive equation obtained from non-linear regression was assessed by the coefficient of determination (R^2) and the root mean square error (RMSE). The former is considered as an overall measure of the precision of the prediction achieved, and it measures the fraction of the variation from the mean that is explained by the model. The later measures the differences between the values predicted by the model, and the values actually observed, and it provides a more intuitive measure of how well the data fits the model. Additionally, predictive values were plotted against observed values to determine data group trends.

9.3 Results

9.3.1 Growth curves

Some growth curves obtained from the TVC and turbidity measurement are listed in appendix A1.6.

9.3.2 Primary model

9.3.2.1 Prediction of the growth kinetic parameter for *L. monocytogenes* and *S. Typhimurium* from TVC by using Baranyi and Roberts and Gompertz model.

The effects of incubation temperature on the growth kinetic parameters of *L. monocytogenes* and *S. Typhimurium* obtained from TVC were determined using Baranyi and Roberts model and Gompertz model (Table 9.2).

Differences between the estimated growth parameters were observed between the two models used. The results obtained from Baranyi and Roberts model indicated that both *L. monocytogenes* and *S. Typhimurium* have a higher growth rate (μ_{max}) and shorter lag time (t_{lag}) and doubling time (t_d) at 30 °C. However, for Gompertz model a higher relative growth rate was observed at 4°C for *L. monocytogenes*, whereas for *S. Typhimurium*, higher relative growth rate was achieved at 15°C. The data related to the goodness of the fit such as the residual sum of square (RSS), the root mean square error (RMS) and the coefficient of determination (R^2) indicated that both models fitted well the observed data. For the Baranyi and Roberts model, the RSS and the RMS for both bacterial groups at different temperature conditions were definitely small range between (0.00- 0.03). According to Sutherland et al. (1994), the lower values obtained from RSS, and RMS indicated the closer fit of the model to the data. For Gompertz model, (R^2) values were very high and close to one ($R^2 > 0.98$). As reported by Banja (2010), the higher the R^2 the better is the prediction by model.

Table 9.2: Growth kinetic parameters obtained from Gompertz and Baranyi and Roberts models for *Listeria monocytogenes* and *Salmonella* Typhimurium from TVC.

| Bacterial strain | T(C°) | Gompertz Model | | | | | Baranyi Model (Baranyi and Roberts) | | | | | | |
|-------------------------------|-------|----------------------------|------|-------|-------|----------------|--|--|--------------------------------------|-----------|---------|------|------|
| | | Growth kinetic parameters* | | | | | Growth kinetic parameters** | | | | | | |
| | | A | C | B | M | R ² | N ₀ log CFUml ⁻¹ | N _{max} log CFUml ⁻¹ | Mu _{max} (h ⁻¹) | t-lag (h) | t-d (h) | RSS | RMS |
| <i>Listeria monocytogenes</i> | 30 | 5.03 | 4.51 | 2.04 | 5.12 | 0.99 | 5 | 9.03 | 1.85 | 3.1 | 0.37 | 0.00 | 0.02 |
| | 15 | 4.99 | 4.60 | 10.2 | 21.9 | 0.98 | 5 | 8.6 | 0.38 | 11.75 | 1.82 | 0.00 | 0.01 |
| | 4 | 5.01 | 4.57 | 62.3 | 124.1 | 0.99 | 4.99 | 8.92 | 0.06 | 60.07 | 11.57 | 0.02 | 0.03 |
| <i>Salmonella</i> Typhimurium | 30 | 5.09 | 4.25 | 2.26 | 6.07 | 0.98 | 5.09 | 8.69 | 1.61 | 4.09 | 0.43 | 0.00 | 0.01 |
| | 15 | 4.99 | 4.23 | 12.36 | 31.3 | 0.99 | 4.9 | 8.86 | 0.27 | 18.34 | 2.26 | 0.01 | 0.03 |

* A: Initial cell density (log CFUml⁻¹); C: Final cell density (log CFUml⁻¹), B: Relative growth rate (h⁻¹); M: Time at which growth rate is maximum; R²: Coefficient of determination, * N₀: initial cell density; N_{max}: Final cell density; Mu_{max}: Maximum specific growth rate; t-lag: Lag time; t-d: doubling time; RSS: Residual sum of square; RMS: Root mean square error.

9.3.2.2 Growth parameter estimate obtained from absorbance measurements for *L. monocytogenes* and *S. Typhimurium* at different temperatures, pH levels, and different concentrations of NaCl and spice extracts.

The turbidity measurements obtained from both *L. monocytogenes* and *S. Typhimurium* were fitted to the Baranyi and Roberts model to create the microbial growth curves for primary modelling. The growth kinetic parameter for these microorganisms under different conditions (two temperature levels, three pH levels, three NaCl concentrations and three spice extract types with three levels of concentrations) are illustrated in Tables 9.3 – 9.8.

The data showed no growth for both microorganisms and for cinnamon high, 5.5% NaCl concentration and all pH levels at 15 and 30°C (Tables 9.3 and 9.6). The data could not fit the model for *L. monocytogenes* at 4°C at any combinations of conditions used, and for rosemary extracts at pH 7 and 6 and for all NaCl concentrations at 15 °C incubation temperature (Table 9.7). These data were not included in the kinetic results.

The highest μ_{\max} (h^{-1}), and shorter lag time (t-lag) and doubling time (d-t) for both microorganisms were detected at 30°C, pH 7, 0.5 NaCl and 0% spice extracts concentrations. The control (broth media with inoculum only; no salt or spice added) at different pH levels and at 30 and 15°C showed a higher growth rate when comparing with other treatments.

For treated samples, the highest $\mu_{\max}(\text{h}^{-1})$ for *L. monocytogenes* at 30°C was gained for pH 7, 0.5% NaCl and 0.05 sumac extracts. Whereas, for *S. Typhimurium*, the highest $\mu_{\max}(\text{h}^{-1})$ was reached at pH7, 0.5% NaCl and cinnamon at low concentrations.

Table 9.3: Predictive growth parameters for *Listeria monocytogenes* and *Salmonella* Typhimurium under the different growth conditions at 30° C were determined using Baranyi and Roberts model.

| T (C°) | NaCl (%) | pH | Spice* extracts | <i>Listeria monocytogenes</i> | | | | <i>Salmonella</i> Typhimurium | | | |
|-----------|-------------|----|--------------------|---|--------|------|------|---|--------|------|------|
| | | | | Mu _{max} (h ⁻¹) | t- lag | t-d | RMS | Mu _{max} (h ⁻¹) | t- lag | t-d | RMS |
| 30 | 0 | 7 | C | 1.34 | 3.77 | 0.52 | 0.01 | 1.25 | 3.94 | 0.55 | 0.00 |
| 30 | 0 | 7 | CH | 0.26 | 19.2 | 2.68 | 0.00 | 0.58 | 6.75 | 1.20 | 0.01 |
| 30 | 1 | 7 | CH | 0.26 | 22.7 | 2.64 | 0.01 | 0.54 | 9.21 | 1.28 | 0.01 |
| 30 | 5 | 7 | CH | - | - | - | - | - | - | - | - |
| 30 | 0 | 7 | CM | 0.37 | 16.3 | 1.88 | 0.01 | 0.75 | 6.35 | 0.95 | 0.01 |
| 30 | 1 | 7 | CM | 0.25 | 20.9 | 2.76 | 0.01 | 0.68 | 8.69 | 1.01 | 0.01 |
| 30 | 5 | 7 | CM | - | - | - | - | - | - | - | - |
| 30 | 0 | 7 | CL | 0.54 | 7.94 | 1.29 | 0.01 | 1.18 | 4.00 | 0.59 | 0.01 |
| 30 | 1 | 7 | CL | 0.40 | 12.9 | 1.75 | 0.00 | 1.01 | 6.46 | 0.69 | 0.00 |
| 30 | 5 | 7 | CL | 0.28 | 24.9 | 2.52 | 0.00 | 0.31 | 16.7 | 2.22 | 0.00 |
| 30 | 0 | 6 | C | 0.97 | 4.29 | 0.7 | 0.01 | 0.78 | 4.01 | 0.95 | 0.01 |
| 30 | 0 | 6 | CH | - | - | - | - | 0.03 | -1.27 | 26.3 | 0.01 |
| 30 | 1 | 6 | CH | - | - | - | - | - | - | - | - |
| 30 | 5 | 6 | CH | - | - | - | - | - | - | - | - |
| 30 | 0 | 6 | CM | 0.21 | 22.2 | 3.35 | 0.00 | 0.40 | 12.23 | 1.71 | 0.02 |
| 30 | 1 | 6 | CM | 0.18 | 26.9 | 3.84 | 0.01 | 0.28 | 16.63 | 2.51 | 0.02 |
| 30 | 5 | 6 | CM | - | - | - | - | - | - | - | - |
| 30 | 0 | 6 | CL | 0.47 | 6.80 | 1.46 | 0.02 | 0.68 | 5.35 | 1.02 | 0.01 |
| 30 | 1 | 6 | CL | 0.2 | 6.63 | 3.52 | 0.01 | 0.56 | 6.61 | 1.25 | 0.01 |
| 30 | 5 | 6 | CL | - | - | - | - | - | - | - | - |
| 30 | 0 | 5 | C | 0.61 | 4.60 | 1.14 | 0.02 | 0.59 | 4.71 | 1.18 | 0.01 |
| 30 | 0 | 5 | CH | - | - | - | - | - | - | - | - |
| 30 | 1 | 5 | CH | - | - | - | - | - | - | - | - |
| 30 | 5 | 5 | CH | - | - | - | - | - | - | - | - |
| 30 | 0 | 5 | CM | - | - | - | - | 0.16 | 27.0 | 4.29 | 0.01 |
| 30 | 1 | 5 | CM | - | - | - | - | 0.14 | 30.1 | 4.91 | 0.01 |
| 30 | 5 | 5 | CM | - | - | - | - | - | - | - | - |
| 30 | 0 | 5 | CL | - | - | - | - | 0.37 | 7.23 | 1.87 | 0.01 |
| 30 | 1 | 5 | CL | - | - | - | - | 0.32 | 8.43 | 2.17 | 0.01 |
| 30 | 5 | 5 | CL | - | - | - | - | - | - | - | - |

*Spice extracts (C: Control; CH: Cinnamon high; CM: Cinnamon medium; CL: Cinnamon low; RH: Rosemary high; RM: Rosemary medium; RL: Rosemary low; SH: Sumac high; SM: Sumac medium; SL: Sumac low), Mu_{max}(h⁻¹): Maximum specific growth rate; t-lag: Lag time; t-d: Doubling time; RMS: Residual mean square error ; (-): No growth.

Table 9.4: Predictive growth parameters for *Listeria monocytogenes* and *Salmonella* Typhimurium under the different growth conditions at 30°C were determined using Baranyi and Roberts model.

| T (C°) | NaCl (%) | pH | Spice* extracts | <i>Listeria monocytogenes</i> | | | | <i>Salmonella</i> Typhimurium | | | |
|-----------|-------------|----|--------------------|---|--------|------|------|---|--------|------|------|
| | | | | Mu _{max} (h ⁻¹) | t- lag | t-d | RMS | Mu _{max} (h ⁻¹) | t- lag | t-d | RMS |
| 30 | 0 | 7 | C | 1.34 | 3.77 | 0.52 | 0.01 | 1.25 | 3.94 | 0.55 | 0.00 |
| 30 | 0 | 7 | RH | 0.48 | 4.90 | 1.45 | 0.02 | 0.77 | 5.66 | 0.93 | 0.02 |
| 30 | 1 | 7 | RH | 0.42 | 5.30 | 1.55 | 0.02 | 0.73 | 5.96 | 0.95 | 0.02 |
| 30 | 5 | 7 | RH | 0.25 | 8.52 | 1.61 | 0.01 | 0.34 | 13.1 | 2.02 | 0.02 |
| 30 | 0 | 7 | RM | 0.52 | 4.50 | 0.96 | 0.02 | 0.85 | 5.54 | 0.81 | 0.00 |
| 30 | 1 | 7 | RM | 0.49 | 5.99 | 1.41 | 0.02 | 0.74 | 6.07 | 0.93 | 0.00 |
| 30 | 5 | 7 | RM | 0.33 | 9.56 | 2.26 | 0.01 | 0.48 | 10.9 | 1.44 | 0.01 |
| 30 | 0 | 7 | RL | 0.62 | 4.73 | 1.11 | 0.02 | 0.83 | 5.26 | 0.84 | 0.01 |
| 30 | 1 | 7 | RL | 0.51 | 4.84 | 1.35 | 0.03 | 0.77 | 5.52 | 0.80 | 0.01 |
| 30 | 5 | 7 | RL | 0.46 | 8.68 | 1.24 | 0.01 | 0.54 | 10.2 | 1.28 | 0.01 |
| 30 | 0 | 6 | C | 0.97 | 4.29 | 0.70 | 0.01 | 0.78 | 4.01 | 0.95 | 0.01 |
| 30 | 0 | 6 | RH | 0.33 | 7.97 | 2.09 | 0.01 | 0.41 | 6.85 | 2.23 | 0.02 |
| 30 | 1 | 6 | RH | 0.31 | 10.9 | 2.21 | 0.01 | 0.32 | 4.88 | 2.14 | 0.03 |
| 30 | 5 | 6 | RH | 0.22 | 26.4 | 3.15 | 0.00 | 0.27 | 10.5 | 2.54 | 0.02 |
| 30 | 0 | 6 | RM | 0.41 | 8.03 | 1.71 | 0.01 | 0.58 | 5.39 | 1.19 | 0.01 |
| 30 | 1 | 6 | RM | 0.32 | 8.74 | 2.17 | 0.01 | 0.47 | 6.87 | 1.47 | 0.01 |
| 30 | 5 | 6 | RM | 0.14 | 18.5 | 4.95 | 0.00 | 0.29 | 9.01 | 2.40 | 0.02 |
| 30 | 0 | 6 | RL | 0.47 | 7.60 | 1.48 | 0.01 | 0.62 | 4.73 | 1.11 | 0.02 |
| 30 | 1 | 6 | RL | 0.40 | 8.03 | 1.75 | 0.02 | 0.51 | 4.84 | 1.35 | 0.02 |
| 30 | 5 | 6 | RL | 0.13 | 11.7 | 5.36 | 0.00 | 0.32 | 8.87 | 2.20 | 0.02 |
| 30 | 0 | 5 | C | 0.61 | 4.60 | 1.14 | 0.02 | 0.59 | 4.71 | 1.18 | 0.01 |
| 30 | 0 | 5 | RH | - | - | - | - | 0.47 | 6.2 | 1.48 | 0.01 |
| 30 | 1 | 5 | RH | - | - | - | - | 0.22 | 7.75 | 3.21 | 0.01 |
| 30 | 5 | 5 | RH | - | - | - | - | - | - | - | - |
| 30 | 0 | 5 | RM | 0.16 | 9.09 | 4.24 | 0.00 | 0.48 | 6.28 | 1.44 | 0.01 |
| 30 | 1 | 5 | RM | 0.09 | 23.5 | 7.73 | 0.00 | 0.44 | 7.31 | 1.57 | 0.02 |
| 30 | 5 | 5 | RM | 0.07 | 23.3 | 9.71 | 0.00 | 0.26 | 8.23 | 2.7 | 0.00 |
| 30 | 0 | 5 | RL | 0.33 | 9.34 | 2.09 | 0.00 | 0.59 | 5.42 | 1.17 | 0.03 |
| 30 | 1 | 5 | RL | 0.21 | 10.4 | 3.24 | 0.00 | 0.35 | 7.36 | 1.97 | 0.03 |
| 30 | 5 | 5 | RL | 0.15 | 51.0 | 4.77 | 0.00 | 0.29 | 8.04 | 2.42 | 0.01 |

*Spice extracts (C: Control; RH: Rosemary high; RM: Rosemary medium; RL: Rosemary low, Mu_{max}(h⁻¹): Maximum specific growth rate; t-lag: Lag time; t-d: Doubling time; RMS: Residual mean square error ; (-): No growth.

Table 9.5: Predictive growth parameters for *Listeria monocytogenes* and *Salmonella* Typhimurium under the different growth conditions at 30°C were determined using Baranyi and Roberts model.

| T (C°) | NaCl (%) | pH | Spice* extracts | <i>Listeria monocytogenes</i> | | | | <i>Salmonella</i> Typhimurium | | | |
|-----------|-------------|----|--------------------|------------------------------------|--------|------|------|------------------------------------|--------|------|------|
| | | | | μ_{\max} (h ⁻¹) | t- lag | t-d | RMS | μ_{\max} (h ⁻¹) | t- lag | t-d | RMS |
| 30 | 0 | 7 | C | 1.34 | 3.77 | 0.52 | 0.01 | 1.25 | 3.94 | 0.55 | 0.00 |
| 30 | 0 | 7 | SH | 0.58 | 6.96 | 1.19 | 0.01 | 0.84 | 4.85 | 0.82 | 0.02 |
| 30 | 1 | 7 | SH | 0.37 | 8.05 | 1.87 | 0.01 | 0.60 | 4.84 | 1.16 | 0.02 |
| 30 | 5 | 7 | SH | 0.16 | 13.4 | 4.35 | 0.01 | 0.25 | 15.5 | 2.8 | 0.01 |
| 30 | 0 | 7 | SM | 0.71 | 6.79 | 0.98 | 0.01 | 0.90 | 4.44 | 0.77 | 0.02 |
| 30 | 1 | 7 | SM | 0.54 | 7.67 | 1.29 | 0.01 | 0.73 | 5.13 | 0.95 | 0.02 |
| 30 | 5 | 7 | SM | 0.29 | 13.2 | 2.42 | 0.01 | 0.53 | 13.8 | 1.30 | 0.01 |
| 30 | 0 | 7 | SL | 0.90 | 6.45 | 0.77 | 0.00 | 1.09 | 4.50 | 0.65 | 0.02 |
| 30 | 1 | 7 | SL | 0.76 | 7.31 | 0.91 | 0.00 | 0.83 | 4.66 | 0.84 | 0.02 |
| 30 | 5 | 7 | SL | 0.33 | 12.1 | 2.07 | 0.00 | 0.63 | 9.94 | 1.10 | 0.01 |
| 30 | 0 | 6 | C | 0.97 | 4.29 | 0.70 | 0.01 | 0.78 | 4.01 | 0.95 | 0.01 |
| 30 | 0 | 6 | SH | 0.21 | 1.59 | 3.23 | 0.02 | 0.47 | 4.6 | 1.47 | 0.02 |
| 30 | 1 | 6 | SH | 0.14 | 1.45 | 5.02 | 0.02 | 0.38 | 2.13 | 1.82 | 0.03 |
| 30 | 5 | 6 | SH | - | - | - | - | 0.25 | 12.8 | 2.73 | 0.02 |
| 30 | 0 | 6 | SM | 0.60 | 3.09 | 1.15 | 0.02 | 0.68 | 3.91 | 0.97 | 0.02 |
| 30 | 1 | 6 | SM | 0.48 | 2.81 | 1.44 | 0.02 | 0.46 | 4.22 | 1.50 | 0.02 |
| 30 | 5 | 6 | SM | 0.21 | 16.6 | 3.23 | 0.02 | 0.26 | 10.2 | 2.71 | 0.01 |
| 30 | 0 | 6 | SL | 0.75 | 3.94 | 0.92 | 0.02 | 0.74 | 3.94 | 0.94 | 0.02 |
| 30 | 1 | 6 | SL | 0.55 | 4.89 | 1.26 | 0.02 | 0.53 | 4.24 | 1.30 | 0.02 |
| 30 | 5 | 6 | SL | 0.27 | 9.08 | 2.59 | 0.01 | 0.36 | 7.22 | 1.91 | 0.01 |
| 30 | 0 | 5 | C | 0.61 | 4.60 | 1.14 | 0.02 | 0.59 | 4.71 | 1.18 | 0.01 |
| 30 | 0 | 5 | SH | 0.22 | 2.33 | 3.09 | 0.02 | 0.32 | 2.71 | 2.15 | 0.02 |
| 30 | 1 | 5 | SH | 0.10 | 3.50 | 6.73 | 0.01 | 0.23 | 10.1 | 3.01 | 0.01 |
| 30 | 5 | 5 | SH | 0.09 | 42.3 | 7.32 | 0.00 | 0.11 | 40.4 | 6.11 | 0.01 |
| 30 | 0 | 5 | SM | 0.14 | 2.50 | 4.80 | 0.02 | 0.38 | 5.64 | 1.84 | 0.01 |
| 30 | 1 | 5 | SM | 0.11 | 3.50 | 6.52 | 0.01 | 0.29 | 6.32 | 2.35 | 0.01 |
| 30 | 5 | 5 | SM | 0.08 | 14.7 | 8.33 | 0.00 | 0.18 | 19.3 | 3.83 | 0.00 |
| 30 | 0 | 5 | SL | 0.85 | 4.22 | 0.81 | 0.01 | 0.42 | 6.85 | 1.63 | 0.01 |
| 30 | 1 | 5 | SL | 0.31 | 6.99 | 2.21 | 0.01 | 0.23 | 7.62 | 3.01 | 0.01 |
| 30 | 5 | 5 | SL | 0.14 | 19.1 | 4.91 | 0.01 | 0.19 | 18.6 | 3.74 | 0.01 |

*Spice extracts (C: Control; SH: Sumac high; SM: Sumac medium; SL: Sumac low), μ_{\max} (h⁻¹): Maximum specific growth rate; t-lag: Lag time; t-d: Doubling time; RMS: Residual mean square error ; (-): No growth.

Table 9.6: Predictive growth parameters for *Listeria monocytogenes* and *Salmonella* Typhimurium under different growth condition at 15°C were determined using Baranyi and Roberts model.

| T (C°) | NaCl (%) | pH | Spice extracts | <i>Listeria monocytogenes</i> | | | | <i>Salmonella</i> Typhimurium | | | |
|-----------|-------------|----|-------------------|---|-------|------|------|---|-------|------|------|
| | | | | Mu _{max} (h ⁻¹) | t-lag | t-d | RMS | Mu _{max} (h ⁻¹) | t-lag | t-d | RMS |
| 15 | 0 | 7 | C | 0.54 | 5.96 | 1.28 | 0.02 | 0.51 | 7.71 | 1.37 | 0.02 |
| 15 | 0 | 7 | CH | 0.18 | 25.9 | 3.73 | 0.00 | - | - | - | - |
| 15 | 1 | 7 | CH | 0.12 | 29.5 | 6.23 | 0.01 | 0.19 | 23.0 | 6.18 | 0.01 |
| 15 | 5 | 7 | CH | - | - | - | - | - | - | - | - |
| 15 | 0 | 7 | CM | 0.22 | 16.5 | 4.58 | 0.00 | 0.25 | 12.3 | 4.01 | 0.01 |
| 15 | 1 | 7 | CM | 0.21 | 19.8 | 6.03 | 0.00 | 0.23 | 15.5 | 4.28 | 0.00 |
| 15 | 5 | 7 | CM | 0.08 | 22.2 | 5.51 | 0.00 | 0.15 | 18.5 | 4.56 | 0.01 |
| 15 | 0 | 7 | CL | 0.24 | 3.72 | 2.93 | 0.00 | 0.27 | 3.22 | 2.73 | 0.00 |
| 15 | 1 | 7 | CL | 0.23 | 6.21 | 3.46 | 0.01 | 0.25 | 5.21 | 3.12 | 0.01 |
| 15 | 5 | 7 | CL | 0.18 | 14.9 | 3.81 | 0.00 | 0.19 | 14.1 | 3.73 | 0.00 |
| 15 | 0 | 6 | C | 0.28 | 11.90 | 2.50 | 0.01 | 0.25 | 12.83 | 2.74 | 0.02 |
| 15 | 0 | 6 | CH | 0.07 | 45.36 | 9.75 | 0.00 | 0.11 | 23.01 | 6.18 | 0.01 |
| 15 | 1 | 6 | CH | - | - | - | - | - | - | - | - |
| 15 | 5 | 6 | CH | - | - | - | - | - | - | - | - |
| 15 | 0 | 6 | CM | 0.12 | 28.52 | 5.58 | 0.00 | 0.15 | 18.46 | 4.59 | 0.01 |
| 15 | 1 | 6 | CM | 0.11 | 35.80 | 6.53 | 0.00 | - | - | - | - |
| 15 | 5 | 6 | CM | 0.02 | 104.2 | 44.5 | 0.00 | - | - | - | - |
| 15 | 0 | 6 | CL | 0.24 | 16.44 | 2.95 | 0.00 | 0.25 | 14.11 | 2.73 | 0.00 |
| 15 | 1 | 6 | CL | 0.19 | 18.49 | 3.61 | 0.00 | 0.21 | 16.45 | 3.11 | 0.00 |
| 15 | 5 | 6 | CL | 0.18 | 30.94 | 3.81 | 0.00 | 0.19 | 21.49 | 3.65 | 0.00 |
| 15 | 0 | 5 | C | 0.24 | 14.43 | 2.86 | 0.00 | 0.20 | 12.10 | 3.43 | 0.01 |
| 15 | 0 | 5 | CH | - | - | - | - | - | - | - | - |
| 15 | 1 | 5 | CH | - | - | - | - | - | - | - | - |
| 15 | 5 | 5 | CH | - | - | - | - | - | - | - | - |
| 15 | 0 | 5 | CM | - | - | - | - | - | - | - | - |
| 15 | 1 | 5 | CM | - | - | - | - | - | - | - | - |
| 15 | 5 | 5 | CM | - | - | - | - | - | - | - | - |
| 15 | 0 | 5 | CL | - | - | - | - | - | - | - | - |
| 15 | 1 | 5 | CL | - | - | - | - | - | - | - | - |
| 15 | 5 | 5 | CL | - | - | - | - | - | - | - | - |

For sample key, see Table 9.3.

Table 9.7: Predictive growth parameters for *Listeria monocytogenes* and *Salmonella* Typhimurium under different growth condition at 15°C were determined using Baranyi and Roberts model.

| T (C°) | NaCl (%) | pH | Spice extracts | <i>Listeria monocytogenes</i> | | | | <i>Salmonella</i> Typhimurium | | | |
|-----------|-------------|----|-------------------|-----------------------------------|-------|------|------|-----------------------------------|-------|------|------|
| | | | | μ_{max} (h ⁻¹) | t-lag | t-d | RMS | μ_{max} (h ⁻¹) | t-lag | t-d | RMS |
| 15 | 0 | 7 | C | 0.54 | 5.96 | 1.28 | 0.02 | 0.51 | 7.71 | 1.37 | 0.02 |
| 15 | 0 | 7 | RH | x | x | x | X | 0.14 | 9.79 | 4.81 | 0.00 |
| 15 | 1 | 7 | RH | x | x | x | X | 0.16 | 11.6 | 4.37 | 0.01 |
| 15 | 5 | 7 | RH | x | x | x | X | 0.13 | 26.9 | 5.03 | 0.01 |
| 15 | 0 | 7 | RM | x | x | x | X | 0.17 | 16.6 | 4.06 | 0.02 |
| 15 | 1 | 7 | RM | x | x | x | X | 0.14 | 12.8 | 4.91 | 0.01 |
| 15 | 5 | 7 | RM | x | x | x | X | - | - | - | - |
| 15 | 0 | 7 | RL | x | x | x | X | 0.41 | 6.45 | 1.42 | 0.01 |
| 15 | 1 | 7 | RL | x | x | x | X | 0.38 | 9.03 | 1.83 | 0.00 |
| 15 | 5 | 7 | RL | x | x | x | X | 0.35 | 10.9 | 1.99 | 0.01 |
| 15 | 0 | 6 | C | 0.28 | 11.9 | 2.5 | 0.01 | 0.25 | 12.8 | 2.74 | 0.02 |
| 15 | 0 | 6 | RH | x | x | x | 0.00 | 0.12 | 11.6 | 5.67 | 0.01 |
| 15 | 1 | 6 | RH | x | x | x | 0.00 | 0.11 | 13.8 | 6.04 | 0.00 |
| 15 | 5 | 6 | RH | x | x | x | - | - | - | - | - |
| 15 | 0 | 6 | RM | x | x | x | 0.00 | 0.17 | 9.79 | 4.06 | 0.02 |
| 15 | 1 | 6 | RM | x | x | x | 0.00 | 0.14 | 12.8 | 4.91 | 0.01 |
| 15 | 5 | 6 | RM | x | x | x | - | - | - | - | - |
| 15 | 0 | 6 | RL | x | x | x | 0.00 | 0.31 | 8.60 | 4.01 | 0.01 |
| 15 | 1 | 6 | RL | x | x | x | 0.00 | 0.27 | 9.89 | 5.11 | 0.00 |
| 15 | 5 | 6 | RL | x | x | x | 0.00 | 0.25 | 17.1 | 5.98 | 0.01 |
| 15 | 0 | 5 | C | 0.24 | 14.4 | 2.86 | 0.00 | 0.20 | 12.1 | 3.43 | 0.01 |
| 15 | 0 | 5 | RH | 0.02 | 31.3 | 34.3 | 0.00 | 0.13 | 12.53 | 5.22 | 0.01 |
| 15 | 1 | 5 | RH | 0.01 | 38.3 | 57.5 | 0.00 | 0.11 | 15.42 | 6.29 | 0.02 |
| 15 | 5 | 5 | RH | - | - | - | - | - | - | - | - |
| 15 | 0 | 5 | RM | 0.16 | 7.18 | 6.78 | 0.00 | 0.22 | 11.32 | 3.12 | 0.02 |
| 15 | 1 | 5 | RM | 0.17 | 24.6 | 4.01 | 0.00 | 0.18 | 12.74 | 4.83 | 0.01 |
| 15 | 5 | 5 | RM | - | - | - | - | - | - | - | - |
| 15 | 0 | 5 | RL | 0.06 | 5.24 | 12.6 | 0.00 | 0.27 | 9.58 | 2.97 | 0.00 |
| 15 | 1 | 5 | RL | 0.13 | 14.4 | 5.17 | 0.00 | 0.24 | 16.7 | 3.84 | 0.00 |
| 15 | 5 | 5 | RL | 0.03 | 53.6 | 23.7 | 0.00 | 0.22 | 19.8 | 4.94 | 0.00 |

X: data not fitted to the model, for sample key see Table 9.4.

Table 9.8: Predictive growth parameters for *Listeria monocytogenes* and *Salmonella* Typhimurium under different growth condition at 15°C were determined using Baranyi and Roberts model.

| T (C°) | NaCl (%) | pH | Spice extracts | <i>Listeria monocytogenes</i> | | | | <i>Salmonella</i> Typhimurium | | | |
|-----------|-------------|----|-------------------|-----------------------------------|-------|------|------|-----------------------------------|-------|------|------|
| | | | | μ_{max} (h ⁻¹) | t-lag | t-d | RMS | μ_{max} (h ⁻¹) | t-lag | t-d | RMS |
| 15 | 0 | 7 | C | 0.54 | 5.96 | 1.28 | 0.02 | 0.51 | 7.71 | 1.37 | 0.02 |
| 15 | 0 | 7 | SH | 0.05 | -1.02 | 14.6 | 0.04 | 0.14 | 8.62 | 5.01 | 0.02 |
| 15 | 1 | 7 | SH | 0.05 | -17.4 | 13.6 | 0.03 | 0.12 | 9.19 | 6.15 | 0.02 |
| 15 | 5 | 7 | SH | - | - | - | - | 0.12 | 23.2 | 5.99 | 0.01 |
| 15 | 0 | 7 | SM | 0.06 | -13.7 | 12.5 | 0.03 | 0.15 | 2.77 | 4.48 | 0.02 |
| 15 | 1 | 7 | SM | 0.04 | -21.1 | 17.5 | 0.02 | 0.08 | -1.08 | 8.71 | 0.02 |
| 15 | 5 | 7 | SM | 0.03 | 56.2 | 20.1 | 0.01 | 0.10 | 23.9 | 6.70 | 0.02 |
| 15 | 0 | 7 | SL | 0.07 | 6.32 | 9.64 | 0.00 | 0.16 | 1.50 | 4.34 | 0.02 |
| 15 | 1 | 7 | SL | 0.05 | 6.56 | 14.1 | 0.02 | 0.15 | 3.13 | 4.20 | 0.02 |
| 15 | 5 | 7 | SL | 0.17 | 25.3 | 4.02 | 0.00 | 0.12 | 19.0 | 5.87 | 0.02 |
| 15 | 0 | 6 | C | 0.28 | 11.9 | 2.5 | 0.01 | 0.25 | 12.8 | 2.74 | 0.02 |
| 15 | 0 | 6 | SH | 0.18 | 3.32 | 3.76 | 0.01 | 0.18 | 8.27 | 3.87 | 0.01 |
| 15 | 1 | 6 | SH | 0.06 | 27.9 | 21.9 | 0.00 | 0.06 | 18.5 | 11.0 | 0.01 |
| 15 | 5 | 6 | SH | - | - | - | - | - | - | - | - |
| 15 | 0 | 6 | SM | 0.34 | 5.13 | 2.02 | 0.02 | 0.17 | 5.09 | 4.13 | 0.02 |
| 15 | 1 | 6 | SM | 0.17 | 4.20 | 3.99 | 0.02 | 0.08 | 16.5 | 10.1 | 0.01 |
| 15 | 5 | 6 | SM | - | - | - | - | - | - | - | - |
| 15 | 0 | 6 | SL | 0.47 | 2.57 | 1.49 | 0.03 | 0.29 | 4.64 | 4.35 | 0.01 |
| 15 | 1 | 6 | SL | 0.41 | 3.32 | 1.68 | 0.02 | 0.16 | 16.6 | 7.37 | 0.01 |
| 15 | 5 | 6 | SL | 0.08 | 5.98 | 8.60 | 0.01 | - | - | - | - |
| 15 | 0 | 5 | C | 0.24 | 14.4 | 2.86 | 0.00 | 0.20 | 12.1 | 3.43 | 0.01 |
| 15 | 0 | 5 | SH | 0.17 | 14.3 | 4.09 | 0.00 | - | - | - | - |
| 15 | 1 | 5 | SH | 0.11 | 22.3 | 6.55 | 0.00 | - | - | - | - |
| 15 | 5 | 5 | SH | - | - | - | - | - | - | - | - |
| 15 | 0 | 5 | SM | 0.12 | 10.7 | 3.24 | 0.01 | - | - | - | - |
| 15 | 1 | 5 | SM | 0.21 | 9.95 | 3.31 | 0.01 | - | - | - | - |
| 15 | 5 | 5 | SM | 0.14 | 15.6 | 4.98 | 0.00 | - | - | - | - |
| 15 | 0 | 5 | SL | 0.16 | 4.37 | 4.39 | 0.02 | 0.14 | 17.2 | 4.93 | 0.01 |
| 15 | 1 | 5 | SL | 0.16 | 6.79 | 4.44 | 0.01 | - | - | - | - |
| 15 | 5 | 5 | SL | 0.1 | 6.06 | 6.70 | 0.01 | - | - | - | - |

For sample key see Table 9.5.

9.3.3 Secondary model

The maximum growth rate ($\mu_{\max} \text{ h}^{-1}$) obtained from the primary model for both *L. monocytogenes*, and *S. Typhimurium* was used to build a response surface analysis model which described the variation of the maximum growth rate as a function of the growth conditions, using a polynomial quadratic function.

The following equations were obtained, consider only the most significant terms for *L. monocytogenes* treated with cinnamon, rosemary and sumac extracts:

For cinnamon extracts (C)

$$\begin{aligned} \mu_{\max} = & 0.1 * \text{temp} - 0.081 * \text{NaCl} + 0.08 * \text{pH} - 0.17 * C + 0.19 * C^2 - \\ & 0.03 \text{ temp} * \text{NaCl} + 0.01 \text{ temp} * \text{pH} - 0.102 \text{ temp} * C - 0.02 \text{ NaCl} * \text{pH} + \\ & 0.02 \text{ NaCl} * C - 0.06 \text{ pH} * C \dots\dots\dots (9.3) \end{aligned}$$

For rosemary extracts (R)

$$\begin{aligned} \mu_{\max} = & 2.01 + 0.11 * \text{temp} + 0.11 * \text{NaCl} - 0.76 * \text{pH} - 0.43 * R + 0.08 * \\ & \text{pH}^2 + 0.14 * R^2 - 0.01 \text{ temp} * R - 0.03 \text{ NaCl} * \text{pH} + 0.03 \text{ NaCl} * R \dots\dots\dots (9.4) \end{aligned}$$

For sumac extracts (S)

$$\begin{aligned} \mu_{\max} = & -0.05 * \text{temp} + 0.01 * \text{NaCl}^2 - 0.04 * \text{pH}^2 + 0.1 * S^2 - \\ & 0.002 \text{ temp} * \text{NaCl} + 0.013 \text{ temp} * \text{pH} - 0.01 \text{ temp} * S - 0.015 \text{ NaCl} * \text{pH} + \\ & 0.03 \text{ NaCl} * S - 0.04 \text{ pH} * S \dots\dots\dots (9.5) \end{aligned}$$

The above equations estimated the predicted growth rate of *L. monocytogenes* in BHI broth under different combinations of conditions (Temperature, pH, NaCl, and spice extracts) (Figures 9.1–9.6).

The regression equation obtained from the response surface analysis model for the maximum growth rate of *L. monocytogenes* under a different combination of conditions revealed that the temperature, pH, NaCl, spice extracts as well as their interaction had significantly affected the growth rate of *L. monocytogenes* in BHI broth ($P < 0.001$).

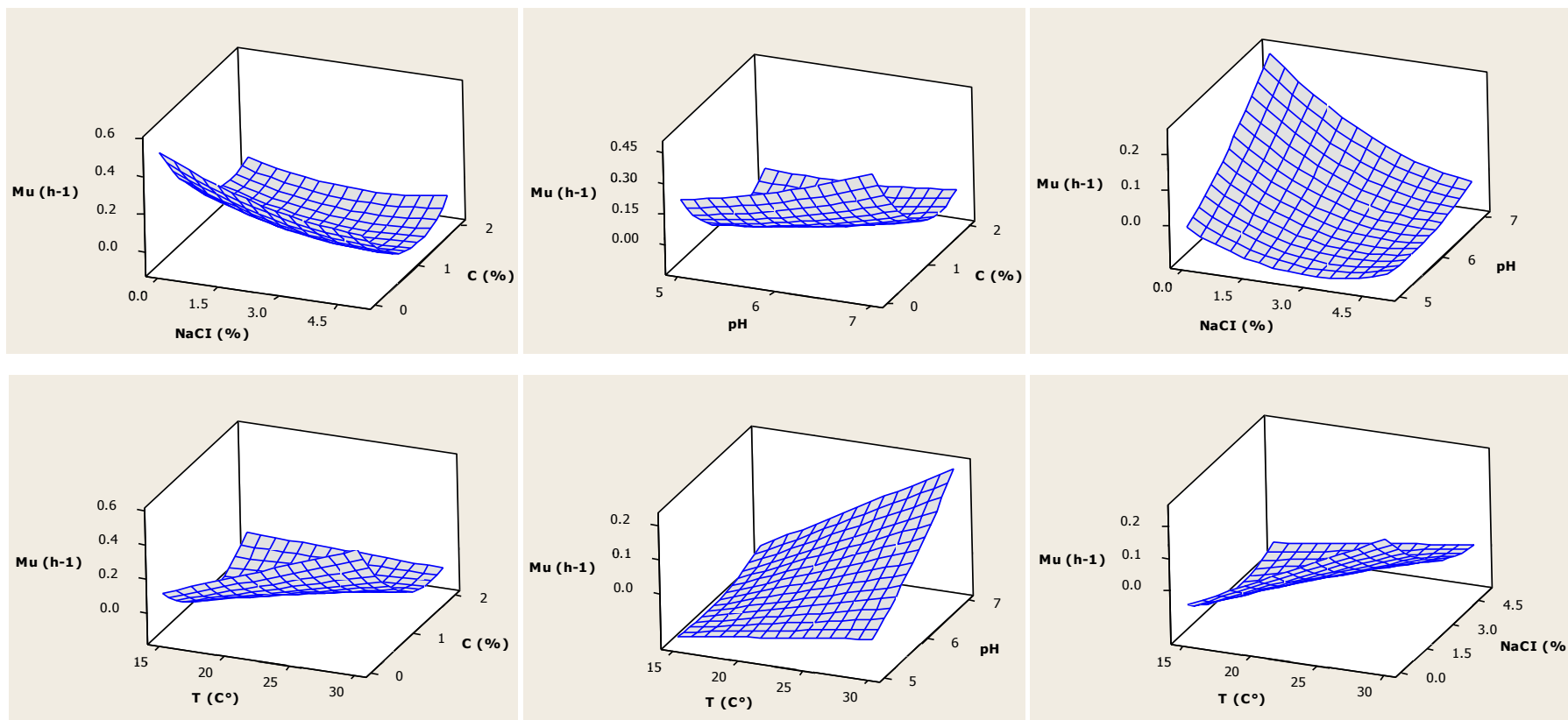


Figure 9.1: Predicted growth rate of *L. monocytogenes* as a function of combinations of NaCl, pH, temperature and cinnamon (C) using response surface analysis model.

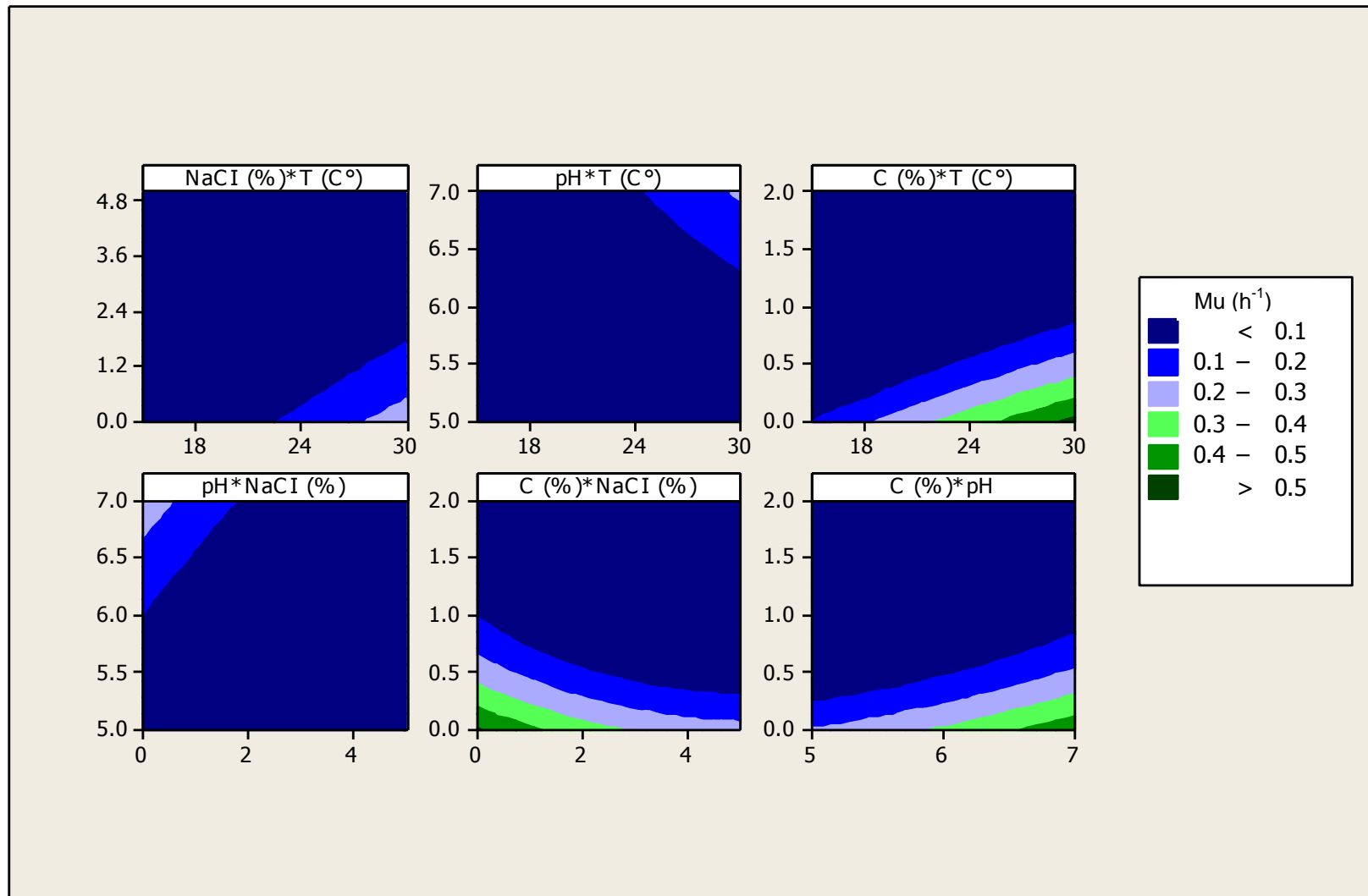


Figure 9.2: Contour plots showed the predicted growth rate of *L. monocytogenes* as a function of combinations of NaCl, pH, temperature (T) and cinnamon(C) using response surface analysis model. Axis labels (Y & X) above each graph.

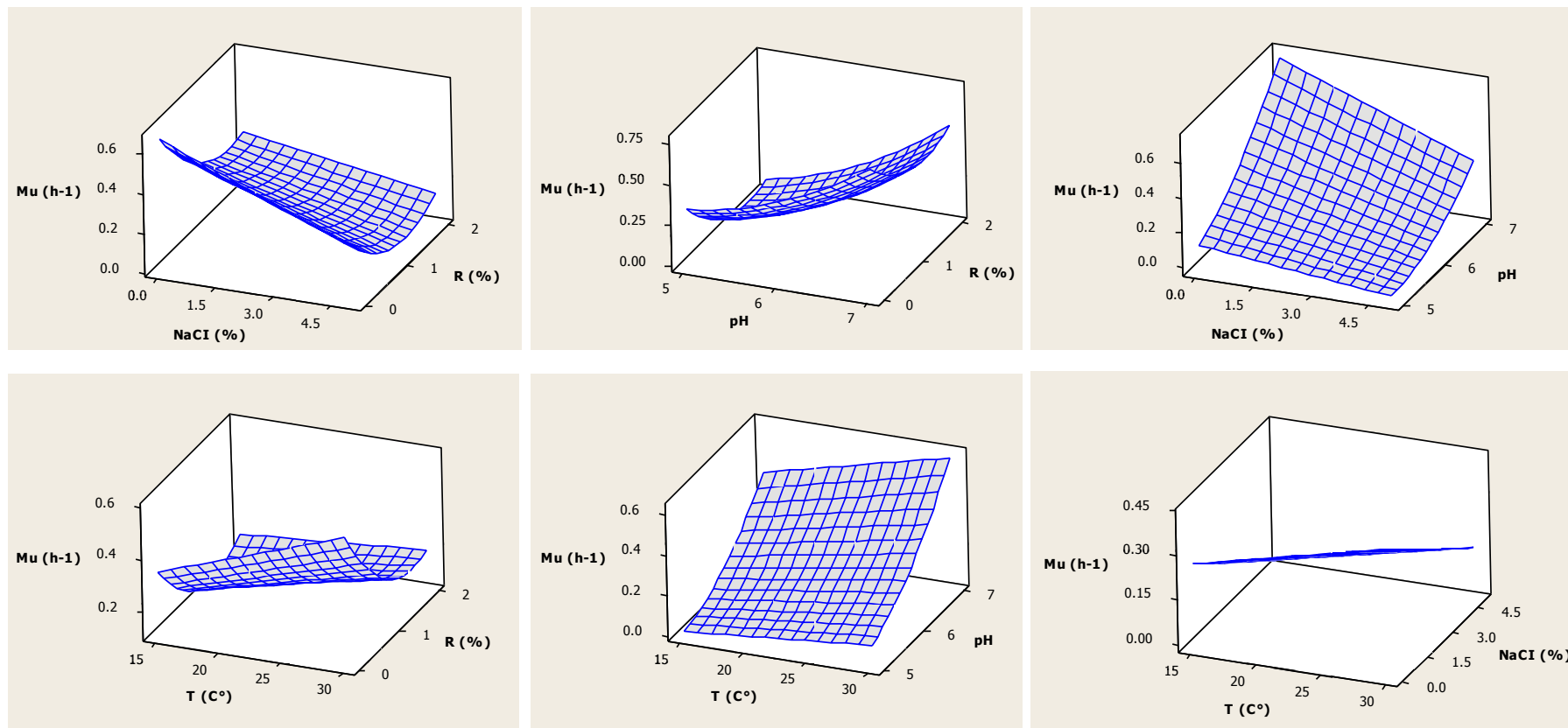


Figure 9.3: Predicted growth rate of *L. monocytogenes* as a function of combinations of NaCl, pH, temperature and rosemary (R) using response surface analysis model.

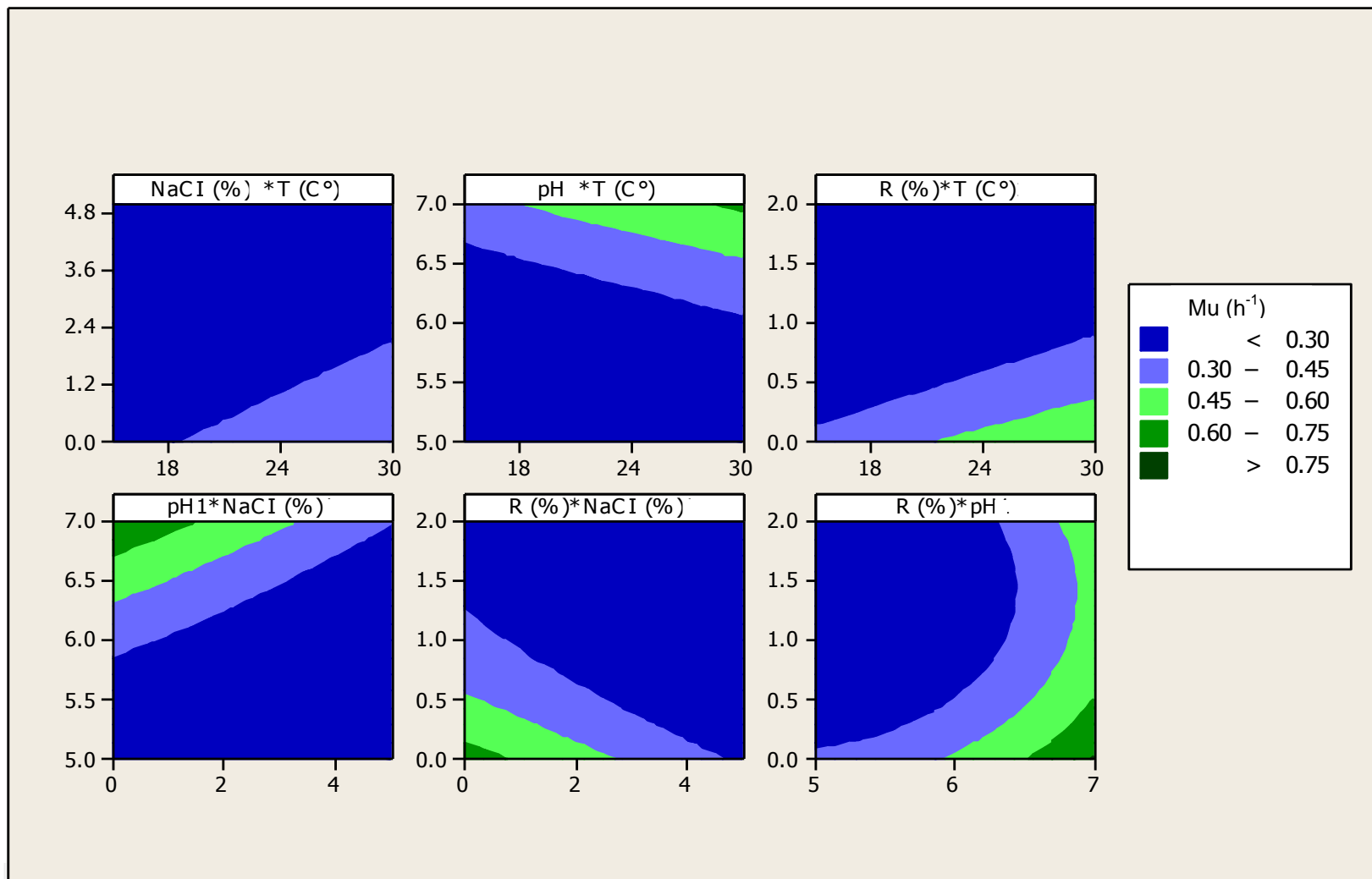


Figure 9.4: Contour plots showed the predicted growth rate of *L. monocytogenes* as a function of combinations of NaCl, pH, temperature (T) and rosemary (R) using response surface analysis model. Axis labels (Y & X) above each graph.

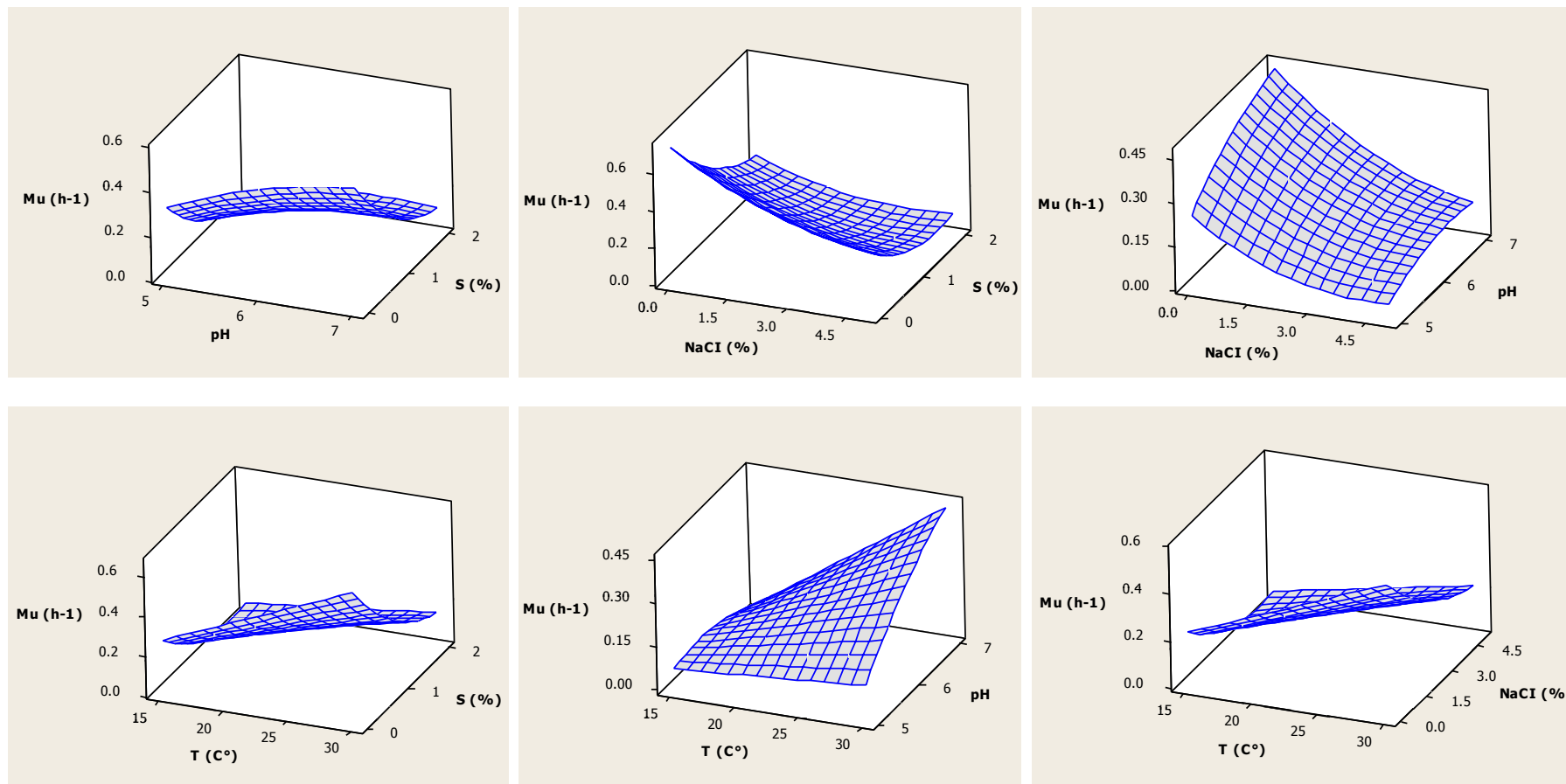


Figure 9.5: Predicted growth rate of *L. monocytogenes* as a function of combinations of NaCl, pH, temperature and sumac (S) using response surface analysis model.

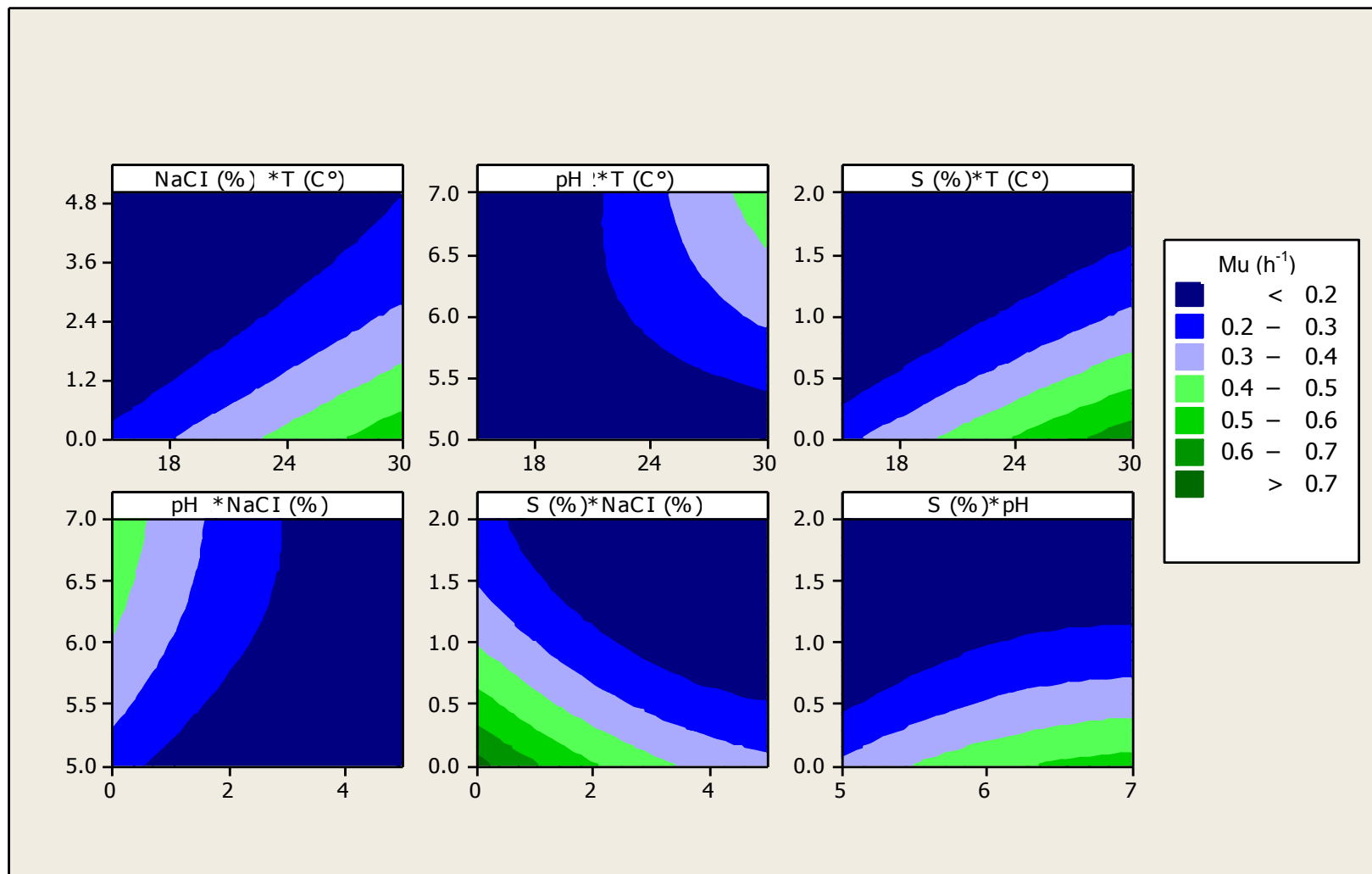


Figure 9.6: Contour plots showed the predicted growth rate of *L. monocytogenes* as a function of combinations of NaCl, pH, temperature (T) and sumac (S) using response surface analysis model. Axis labels (Y & X) above each graph.

For *S. Typhimurium* the following equations were obtained:

For Cinnamon extracts (C)

$$\begin{aligned} Mu_{max} = & 2.4 - 0.04 * temp + 0.23 * NaCl - 0.9 * pH + 0.35 * C + \\ & 0.063 pH^2 - 0.01 temp * NaCl + 0.02 temp * pH - 0.02 temp * C - \\ & 0.03 NaCl * pH + 0.03 NaCl * C - 0.03 pH * C \dots\dots\dots (9.6) \end{aligned}$$

For Rosemary extracts (R)

$$\begin{aligned} Mumax = & 1.9 - 0.02 * temp + 0.06 * NaCl - 0.73 * pH + 0.13 * R + 0.01 * \\ & NaCl^2 + 0.048 * pH^2 - 0.004 temp * NaCl + 0.012 temp * pH - 0.008 temp * \\ & R - 0.006 NaCl * pH \dots\dots\dots (9.7) \end{aligned}$$

For sumac extracts (S)

$$\begin{aligned} Mumax = & 0.98 - 0.04 * temp + 0.06 * NaCl - 0.41 * pH + 0.31 * S + \\ & 0.02 NaCl^2 + -0.04 temp * NaCl + 0.02 temp * pH - 0.01 temp * S - \\ & 0.01 NaCl * pH - 0.03 pH * S \dots\dots\dots (9.8) \end{aligned}$$

The regression equations of the response surface model for $Mu_{max}(h^{-1})$ of *S. Typhimurium* against temperature, NaCl concentrations, pH and spice extracts show that all these factors, and their interactions were significantly affecting the growth rate of *S. Typhimurium* in BHI broth ($P < 0.001$) (Figures 9.7- 9.12).

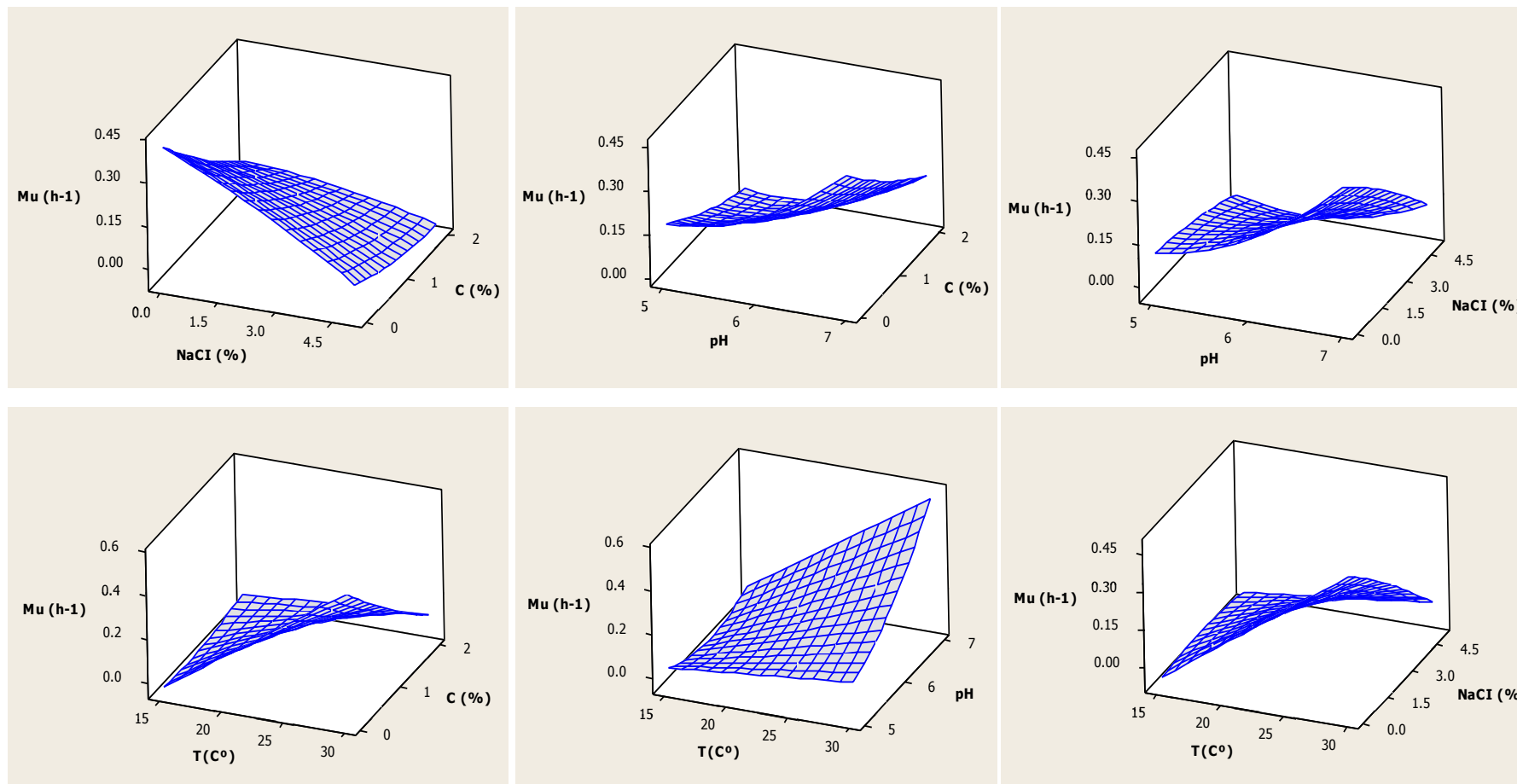


Figure 9.7: Predicted growth rate of *S. Typhimurium* as a function of combinations of NaCl, pH, temperature and cinnamon (C) using response surface analysis model.

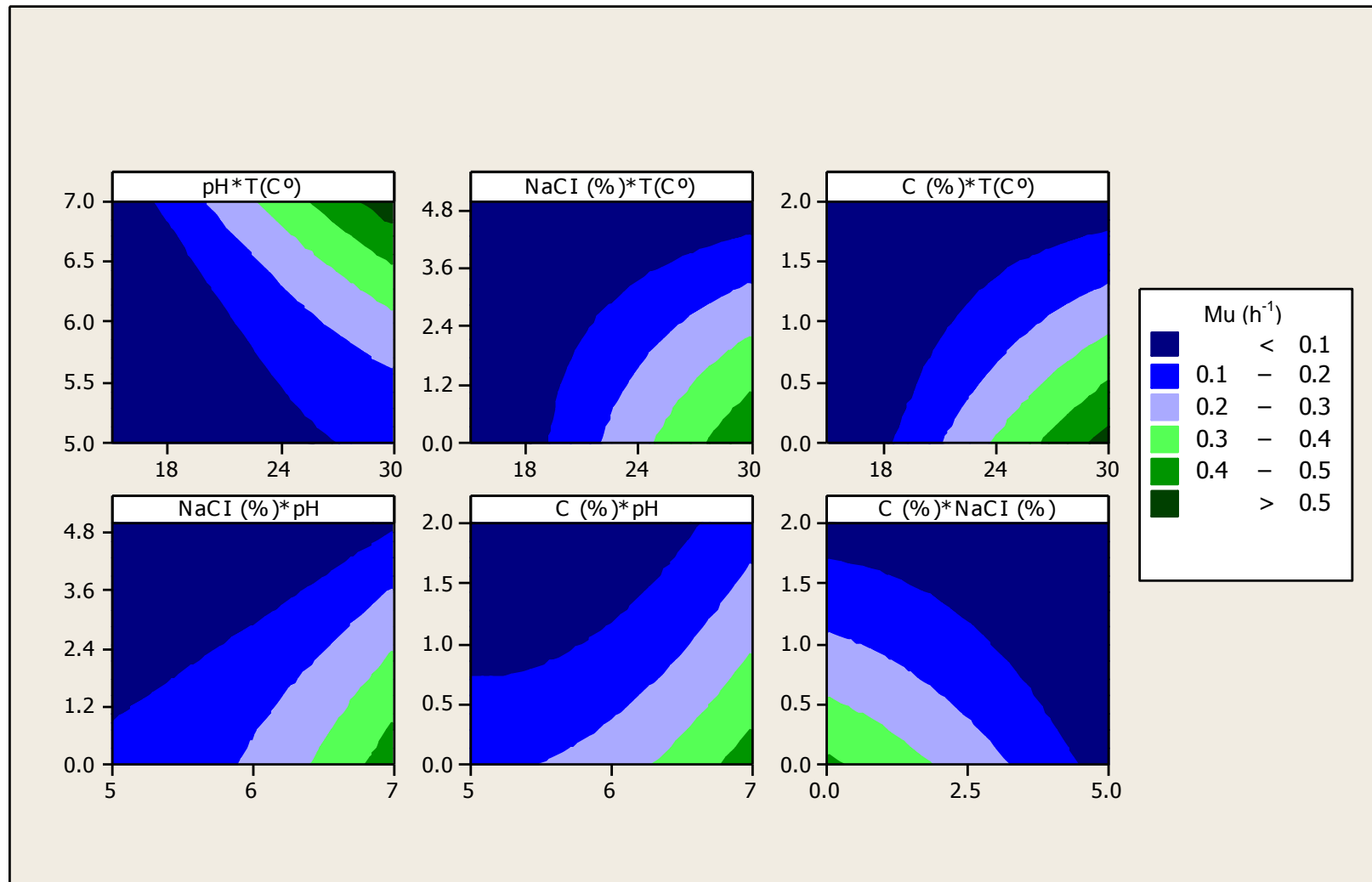


Figure 9.8: Contour plots showed the predicted growth rate of *S. Typhimurium* as a function of combinations of NaCl, pH, temperature (T) and cinnamon (C) using response surface analysis model. Axis labels (Y & X) above each graph.

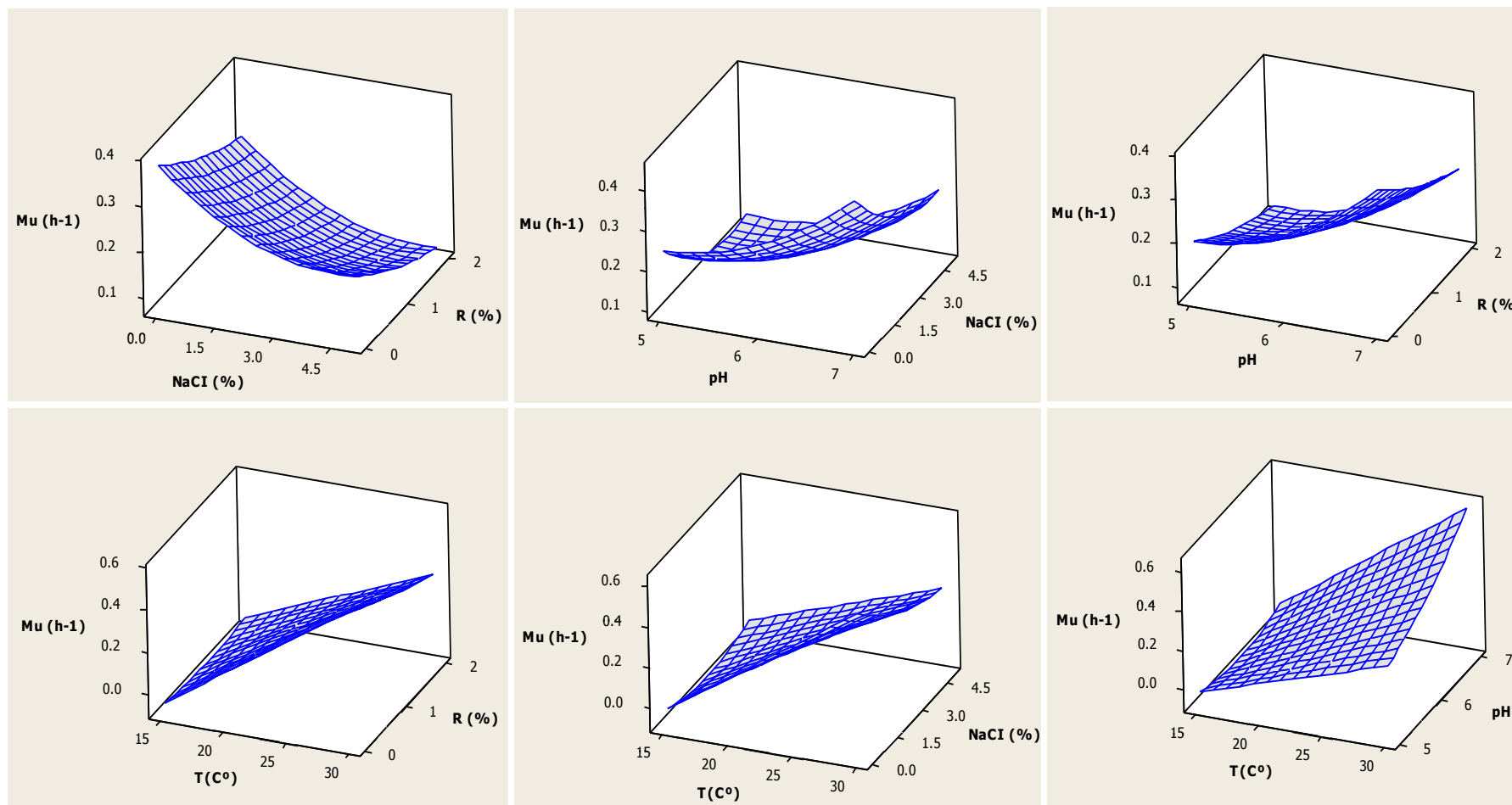


Figure 9.9: Predicted growth rate of *S. Typhimurium* as a function of combinations of NaCl, pH, temperature and rosemary (R) using response surface analysis model.

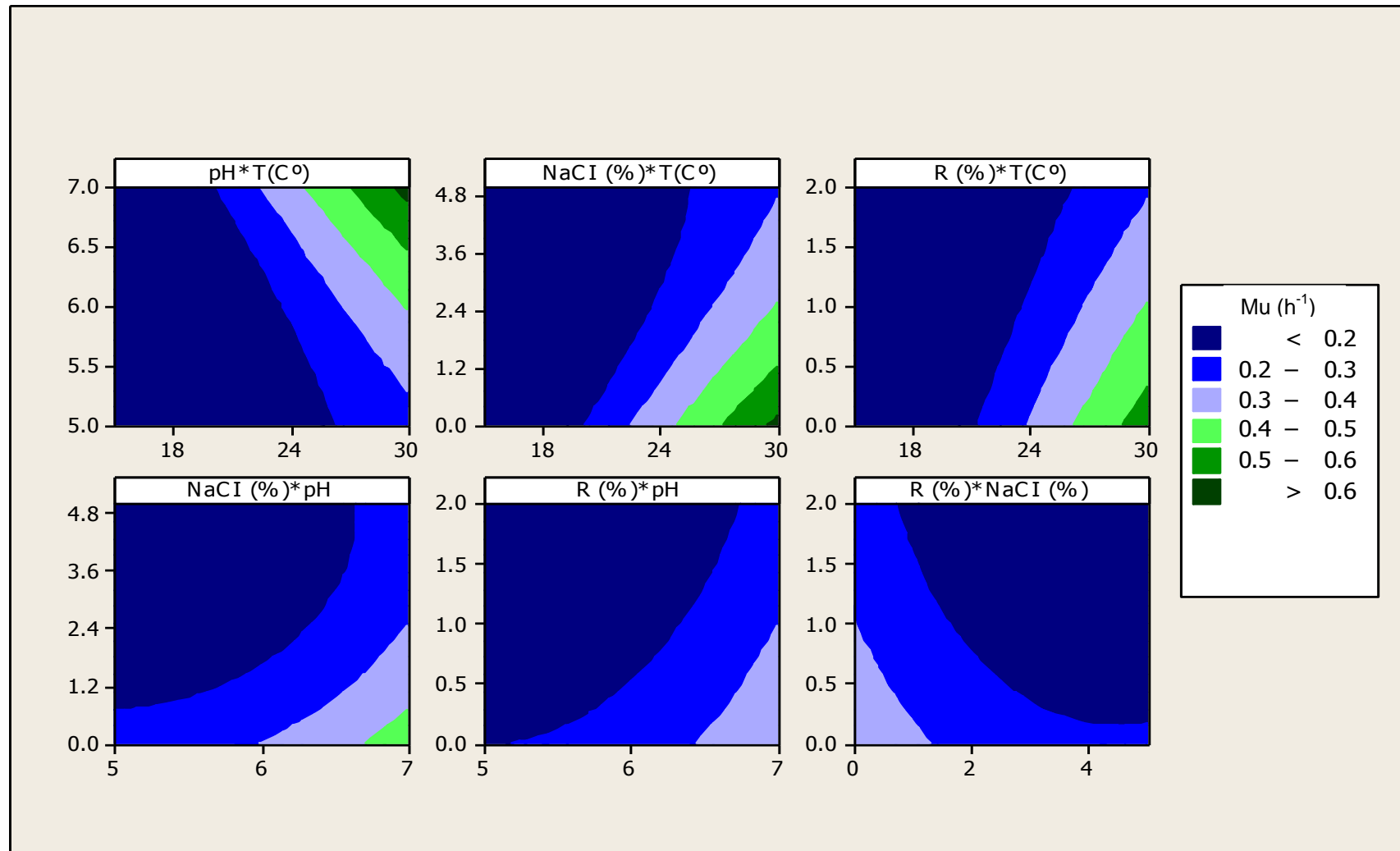


Figure 9.10: Contour plots showed the predicted growth rate of *S. Typhimurium* as a function of combinations of NaCl, pH, temperature (T) and rosemary (R) using response surface analysis model. Axis labels (Y & X) above each graph.

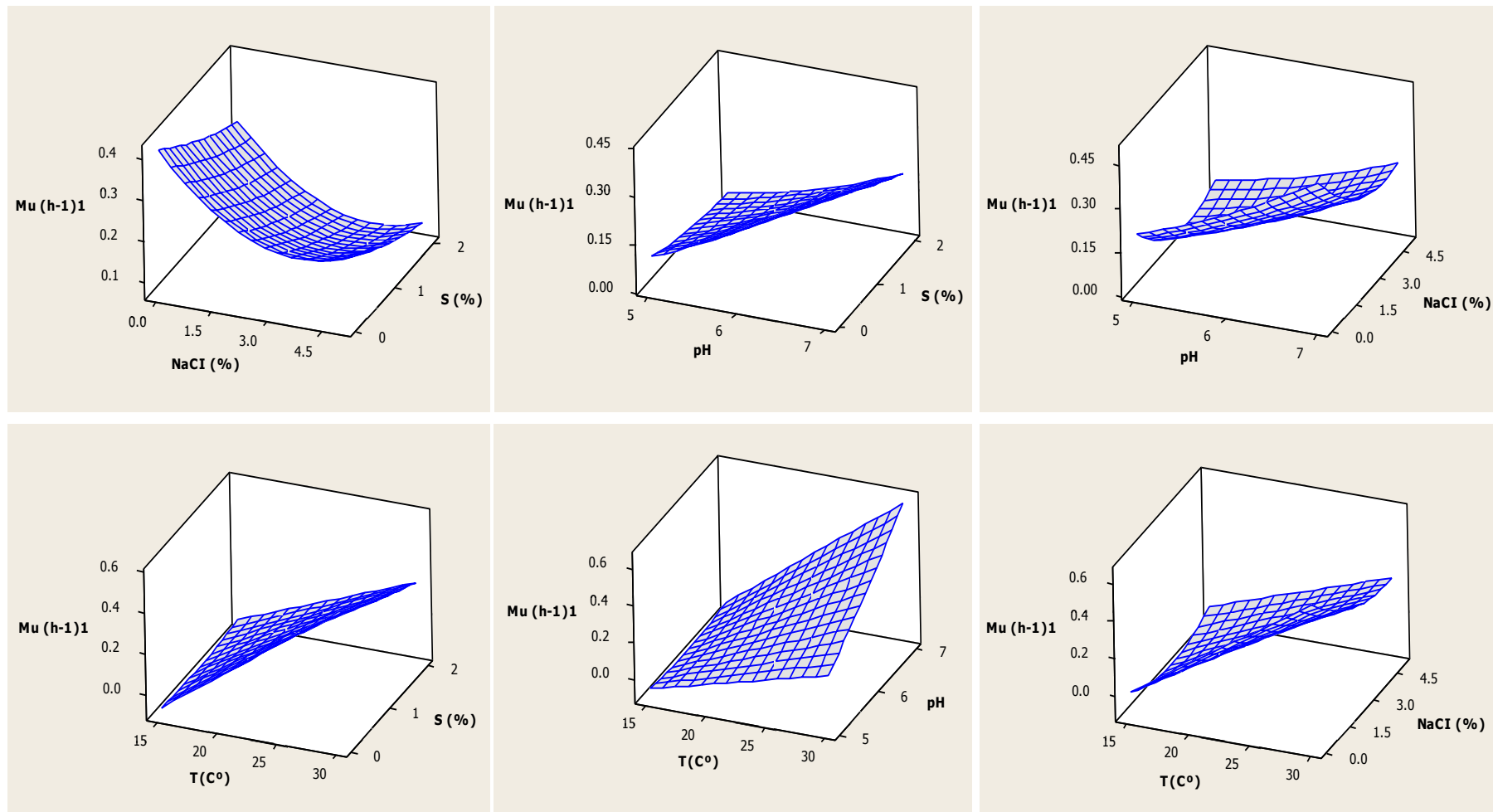


Figure 9.11: Predicted growth rate of *S. Typhimurium* as a function of combinations of NaCl, pH, temperature and sumac (S) using response surface analysis model.

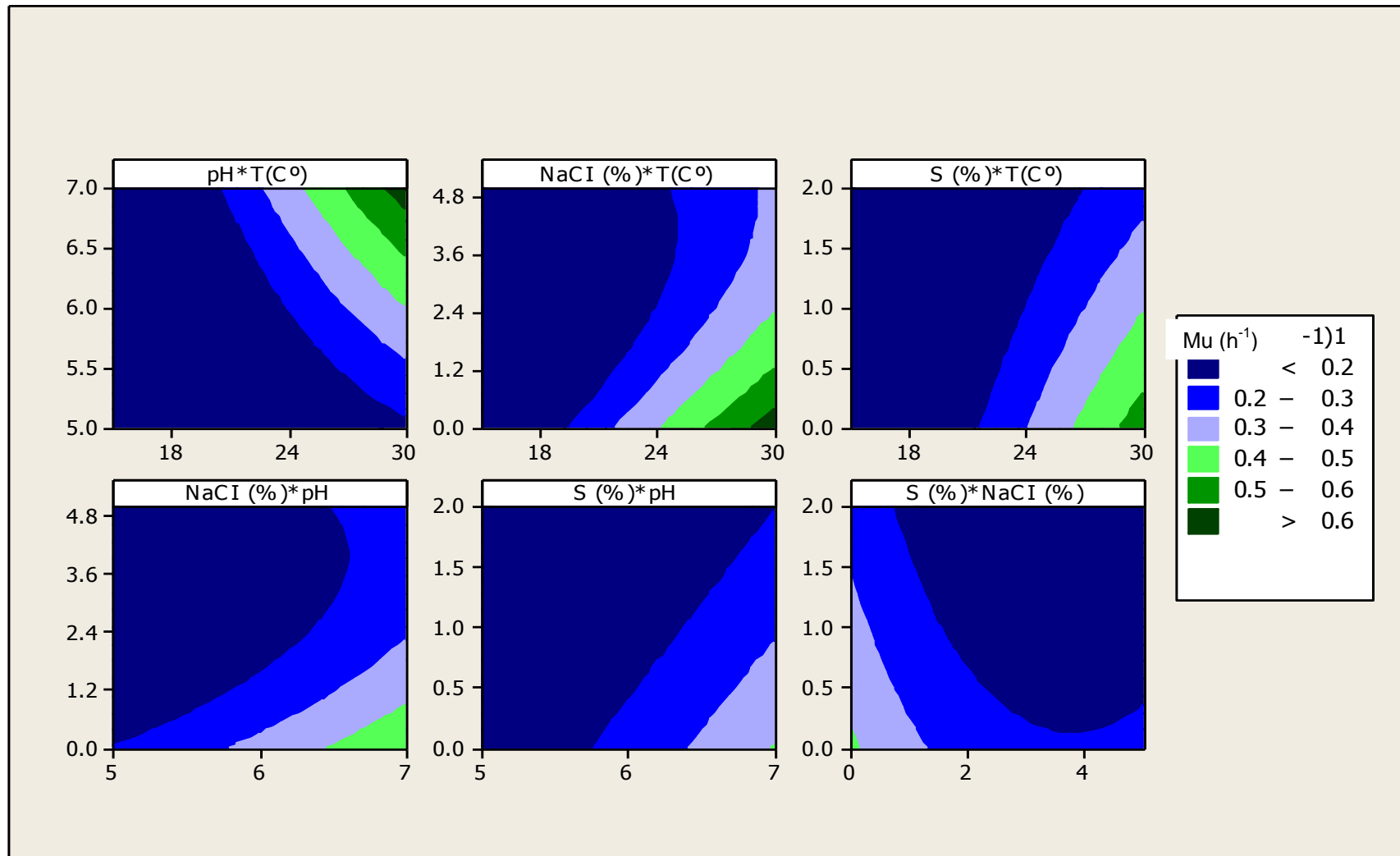


Figure 9.12: Contour plots showed the predicted growth rate of *S. Typhimurium* as a function of combinations of NaCl, pH, temperature (T) and sumac (S) using response surface analysis model. Axis labels (Y & X) above each graph.

9.3.3.1 Evaluation of the models performance

The evaluation of the model performance on the combined effect of temperature, pH, NaCl and spice extracts in the prediction of *L. monocytogenes* and *S. Typhimurium* in BHI broth are presented in Table 9.9.

The statistical indices of R^2 and the root mean square errors RMSE were used to indicate model performance. The R^2 values for the model was above 0.83 for prediction the growth rate of *L. monocytogenes* under combined effects of temperature, pH, NaCl and spice extracts treatments, whereas the R^2 values of more than 0.93 was attended for prediction the growth rate of *S. Typhimurium*. The root mean square errors (RMSE) were very small for both bacterial groups (Table 9.9).

Table 9.9: Evaluation of response surface model performance on the combined effect of temperature, pH, NaCl and different spice extracts in the prediction of *Listeria monocytogenes* and *Salmonella Typhimurium*.

| Spice treatments | <i>L. monocytogenes</i> | | <i>S. Typhimurium</i> | |
|------------------|-------------------------|-------|-----------------------|-------|
| | R^2 | RMSE | R^2 | RMSE |
| Cinnamon | 0.83 | 0.015 | 0.93 | 0.007 |
| Rosemary | 0.94 | 0.008 | 0.96 | 0.004 |
| Sumac | 0.94 | 0.006 | 0.97 | 0.003 |

R^2 : coefficient of determination, RMSE: root mean square error

Additionally, a graphical comparison was performed by plotting predictive values against observed values (data obtained from Table 9.4 and equation 9.7) to determine data group trends and to exemplify the goodness of fit of the proposed RSM (Figure 9.13).

Figure 9.13 demonstrates how the points fall close to the correspondence line, indicating that data correlation is high ($R^2 = 0.93$), so the proposed models describe the growth rate adequately.

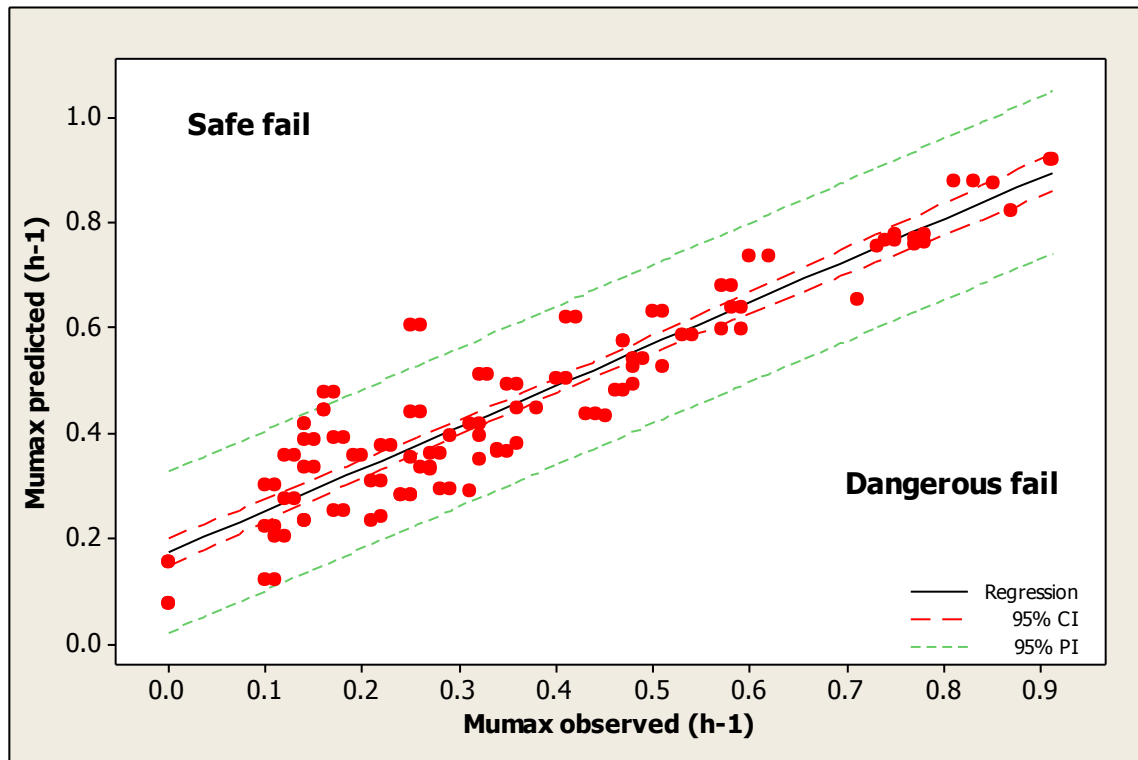


Figure 9.13: Graphical comparison of growth rate observed and growth rate predicted by using response surface analysis model for *S. Typhimurium* as a function of combinations of NaCl, pH, temperature and rosemary extracts

9.4 Discussion

Mathematical models are promising tool to predict the growth of pathogenic and spoilage bacteria in food, to test or build models it is always necessary to measure the growth curves of bacteria. In this study, both primary modelling (Baranyi and Roberts and Gompertz) and secondary modelling (response surface analysis) were used to describe the effects of environmental conditions (temperature, pH and NaCl) and spice extracts on the changes in growth rate for *L. monocytogenes* and *S. Typhimurium*.

9.4.1 Predict the growth kinetic parameter for *L. monocytogenes* and *S. Typhimurium* from TVC by using Baranyi and Roberts and Gompertz model

The results obtained from primary models of the TVC for *L. monocytogenes* and *S. Typhimurium* have shown that Baranyi and Roberts model is consistent and appropriate for obtaining precise estimates of microbial growth kinetic (Table 9.2). Whereas, Gompertz model gave un realistic parameter with higher estimated growth rate for both bacterial groups, corroborating what other authors have reported (Membr'e et al. 1999, Augustin and Carlier 2000). According to Robin and McKellar (2005), the Gompertz growth rate (μ_{\max}) is always the maximum rate and occurs at an arbitrary point of inflection, thus some limitations are associated with the use of this model. Graham et al.(1996) reported that the Gompertz model fits a sigmoid function with a pronounced inflexion and hence tends to overestimate the growth rate, while the Baranyi model has an approximately straight line in the exponential phase, thus it avoided over estimation of μ_{\max} inherent in Gompertz model.

Baranyi and Roberts model showed higher μ_{\max} (h^{-1}) values for both bacterial groups at 30°C , which is agreed with previously reported data (Augustin and

Carlier 2000), whereas, the Gompertz model has shown the higher μ_{\max} (h^{-1}) values at low temperatures for both *L. monocytogenes* and *S. Typhimurium*. This variation in the μ_{\max} (h^{-1}) values for both bacterial strains, especially at lower temperature is definitely due to different types of model used. Gompertz model fits a sigmoid function when growth is slowest at the start and end of a time period (McDonald and Sun 1999), however the Baranyi model proposed a dynamic model for the prediction microbial growth, in conjunction with an adjustment function that depends on the physiological status of microbial cells (Baranyi et al. 1993a). In agreement with our results, Augustin and Carlier (2000) reported that differences between the estimated growth parameters of *L. monocytogenes* were due to the use of different types of model. Additionally they found that the Gompertz function led to a significant and great over-estimation of μ_{\max} (h^{-1}) values.

The calculated t-lag (h) parameter from the Baranyi and Roberts model was found to be temperature dependent for both bacterial groups. Longer t-lag (h) was observed at lower temperature, while on the contrary shorter t-lag (h) was obtained at higher temperatures. The Baranyi and Roberts model expressed the duration of the lag period t-lag (h) as the time required to carry out the work necessary for the cells to overcome an initial hurdle (h_0) and to get ready to divide, which requires adaptation work (Baranyi et al. 1993b, Baranyi and Roberts 1994). Current results indicated that the work to be done for a given temperature increased when the final temperature decreased, which is in line with the data obtained from Muñoz-Cuevas et al. (2010).

The longer doubling time ($t-d \text{ h}^{-1}$) attained at lower temperature for both bacterial groups, was related to the increased in lag phase durations as suggested by (Buchanan and Klawitter 1992).

The statistical indices represented by the residual sum of squares (RSS) and root mean squares error (RMS) values obtained from Baranyi and Roberts model were very small. As stated by Sutherland et al (1994), the lower the value of RMS, the better is the adequacy of the model to describe the data.

The high values of the coefficient of determination (R^2) developed from Gompertz model are an indication of a better fit of the model. As reported by Grau and Vanderlinde (1993), the higher the R^2 value, the better is the prediction by the model.

Depending on the above statements, these results showed that Baranyi and Roberts model demonstrated a reliable prediction of the growth kinetic parameter for both bacterial groups.

9.4.2 Growth parameter estimate obtained from absorbance measurement for *L. monocytogenes* and *S. Typhimurium* at different temperatures, pH levels, and various concentrations of NaCl and spice extracts.

Data obtained from the turbidity measurements for both bacterial groups at different growth conditions (temperatures, pH levels, and different concentration of NaCl and spice extracts) were fitted to the Baranyi and Roberts model; in addition the data that showed no growth or did not fit the Baranyi and Roberts model were excluded from this study (Tables 9.3 - 9.8).

For the data that did not fit Baranyi and Roberts model, it is suspected that the main cause for poor fit the model for some of the data is attributed to the issue of condensation on the side of the 48 microplate wells during the absorbance measurement period. The condensation problem is more obvious when the microplate wells were incubated at low temperatures (15 and 4°C). This problem is unavoidable because it interfered with readings and thus generated inconsistent and unrealistic absorbance data.

The growth kinetic estimates showed that both *L. monocytogenes* and *S. Typhimurium* attained higher values for maximum growth rate (μ_{\max} h⁻¹) at 30°C and in control samples than for treated samples. Many studies have been reported that the temperature was the most important factor controlling bacterial growth (Gibson et al. 1988; Mackey and Kerridge 1988; Buchanan et al. 1998; Valero et al. 2007).

The higher μ_{\max} h⁻¹ values in control may be linked to the absence of salt and spice extracts in control samples. The inhibitory effect of salt on the growth rate of both bacterial groups is well documented (Fernández et al. 1997b, Nerbrink et al. 1999, Koutsoumanis et al. 2004b). Additionally, previous results confirmed the antimicrobial effect of spice extracts against both *L. monocytogenes* and *S. Typhimurium* (Al-Kutby et al. 2009), which is in agreement with previously

reported studies (Nasar-Abbas and Halkman 2004, Charu et al. 2008, Tajkarimi et al. 2010, Weerakkody et al. 2010).

The current results showed that t-lag (h) parameter of the model was shorter when the temperature and pH increased. Whilst, higher NaCl and spice extracts concentrations increased the t-lag (h) duration for example *Listeria monocytogenes* showed a t-lag (h) of 29.5 and 6.2 for CH and CL respectively (Table 9.6).

Several factors could influence the t-lag (h) duration such as: history of the cell, stresses, cell physiological status and the current growth environment (Mellefont et al. 2003, Pin and Baranyi 2008). According to Robinson et al. (1998), t-lag (h) represents a transition period when the microbial cells adjust to their new environment. Whereas, Dalgaard (1995) and Mellefont et al. (2003), reported that a t-lag (h) was the amount of work to be done to adjust to new environmental conditions and the rate at which that work is done.

The critical step in evaluating a predictive model; it is to make sure that the model describes precisely the data from which it has been generated. In this study, the RMS was used as a statistical index to evaluate the model performance, which was definitely small for both bacterial groups at various conditions used. The current results corroborate those from other authors (George et al. 1996; McClure et al. 1997; Carrasco et al. 2006), whom had found that the Baranyi and Roberts model was accurately predicted the growth of *L. monocytogenes* as it gave predictions that were very close to the observed values.

9.4.3 Secondary model

The statistical indices represented by R^2 and RMSE obtained from the secondary model are listed in Table 9.9. The R^2 values were relatively high for both bacteria, indicating the adequacy of the data to fit the model. Moreover, RMSE values were definitely small in comparison with previous studies (Buchanan and Phillips 1990, Valero et al. 2006). For example the RMSE obtained from the Response surface model of *L. monocytogenes* was range between 0.015 to 0.006, whereas Valero et al. (2006) reported that the RMSE obtained from the Response surface model of *L. monocytogenes* estimated by absorbance data was 0.0223. Mathematical and graphical comparison Prove that the RS equation obtained describes properly *S. Typhimurium* growth rate (Figure 9.13).

Once the RSM were obtained, the effect of the environmental factors (Temperature, pH, and NaCl) as well as the spices extracts on the growth rate of *L. monocytogenes* and *S. Typhimurium* were analysed with statistical parameters and with the aid of response surface graphs (Figures 9.1- 9.12)

➤ Effect of Temperature

Despite the psychrotrophic features of *L. monocytogenes*, low temperature was the most decisive factor for controlling the rate of growth (Figures 9.1- 9.6). In agreement with the current results, Buchanan et al (1998) and Carrasco et al. (2006), they found that growth rate was increased when the temperature approaches the optimal growth temperature of *L. monocytogenes*. Nevertheless, Nyati (2000), observed that the psychrotrophic nature of *L. monocytogenes* allows it to grow well in ready-to-eat food stored at refrigeration temperatures, and thus may reach levels that may cause illness. This difference could be the result of different models used with different bacterial strains and environmental conditions.

For *S. Typhimurium*, the same effect of temperature was observed. The current results show, corroborating with other authors (Thomas 2002, Basti and Razavilar 2004), that low temperature has a strong inhibition. Beaudry et al.(1992) reported that the biological reactions tend to increase by a factor of two or three for each 10°C increase in temperature.

➤ Effect of pH

It is noteworthy that the lowest pH value decreased the growth rate for both bacteria. According to George et al. (1996) and Gahan and Hill (1999) , *L. monocytogenes* and *S. Typhimurium* are more tolerant to acid than other food-borne pathogens; they grow and survive at pH 4.3 and 3.6 respectively (Giannuzzi and Zaritzky 1996, Garcia 2009). However, the acid resistance was depending on the strain type, growth condition, medium composition and physiological cell state (Phan-Thanh et al. 2000, Koutsoumanis et al. 2004a, Álvarez-Ordóñez et al. 2009). The current results indicated that acid conditions

(pH < 5) produce a strong inhibition, in agreement with other authors (Carrasco et al. 2006, De Keersmaecker et al. 2006, Valero et al. 2006)

➤ Effect of NaCl

As expected, for *L. monocytogenes* and *S. Typhimurium* maximum growth rate (μ_{max}) decreased when the NaCl concentrations approached the higher levels (5%). Previously reported data from Vermeulen et al. (2007) indicated that *L. monocytogenes* were not able to grow at a_w less than 0.93 (10% NaCl) and also they found that a_w had less influence effect on the growth / no growth boundary of *L. monocytogenes* than other inhibitory factor in mayonnaise based salads stored at refrigerated temperature. Our results revealed a significant effect of NaCl ($P < 0.001$) against *L. monocytogenes* in BHI broth using response surface model, corroborating other studies (Boziaris and Nychas 2006, Tiganitas et al. 2009, Karina et al. 2011)

Similarly, *S. Typhimurium* was reported to be considerably affected by increased NaCl concentrations. According to Basti and Razavilar (2004), NaCl affected the growth rate of *S. Typhimurium* in BHI broth and the effectiveness of potassium sorbate on the growth of *S. Typhimurium* was enhanced by addition of NaCl at a given pH. Tiganitas et al. (2009), revealed that higher inactivation of *S. Typhimurium* was observed after exposure to 20% NaCl compared to 15% NaCl in tryptone soy broth.

➤ Spice extracts effects

In general, cinnamon, rosemary and sumac extracts showed antimicrobial effects against both *L. monocytogenes* and *S. Typhimurium*, with the higher effects obtained from the cinnamon extracts. Previous results had demonstrated that cinnamon, rosemary and sumac had antimicrobial effect against *S.*

Typhimurium (Al-Kutby et al. 2009) and *L. monocytogenes* (Al-Kutby et al. 2009, 2010).

In this study, the antimicrobial activity of natural spice extracts was concentration-dependent and can be categorised into three responses: firstly, no bacterial growth. Secondly, increase lag phase duration, which could be due to either bacterial cells damage which need some time to repair before growth, or some cells are killed and only a few survive and then grow; and thirdly, a decrease in the growth rate compared with the control. Finally a combination of the second and third phenomena could occur. Therefore, if the organisms required only adjustment time to repair and multiply normally in the presence of the antibacterial spice extracts, thus only the second effect will be seen. Whereas, if the spice extracts affect the generation time of organisms, then a combination of both second and third responses will be evident. In agreement with this results, Koutsoumanis et al (1998), found that the maximum growth rate of *Salmonella* Enteritidis was significantly lower in samples with oleuropein essential oil, when comparing with control samples. Additionally, they reported that low oleuropein concentration delays the growth of *Salmonella* Enteritidis, whereas high concentration reduced the growth rate and thereby decrease the slope of growth curve.

➤ Combined effects

Combined effects of temperature, pH, NaCl and spice extracts were observed on both bacterial groups. The addition of higher concentration of spice extracts at low temperatures with lowest pH and higher NaCl concentration was more effective in limiting the growth of *L. monocytogenes* and *S* Typhimurium in BHI broth. For example, when the temperature was reduced from 30 to 15 °C, the effect of combinations of rosemary at 0.125%, NaCl 1% and pH 5 was decrease

the growth rate from 0.44 to 0.18 h⁻¹. These results agree with previously reported studies (Carrasco et al. 2006, Valero et al. 2006).

9.5 Conclusion

The model developed in this study could be useful for predicting *L. monocytogenes* and *S. Typhimurium* behaviour in RTE meat products with similar conditions to those used in this work. The different types of model provided different growth information and are thus complementary

The Baranyi and Roberts model has given precise and reliable estimates of growth kinetic parameters for both bacterial groups. the minimum growth rate as obtained from Baranyi and Roberts model are temperature of 15°C, 5.5% NaCl concentration, 0.125 - 0.2 % spice extracts concentration (specially cinnamon), and pH 5.

In this study, spice extracts are shown to be a fundamental factor to take into account during food processing to control pathogens. Cinnamon, rosemary and sumac act as either bactericidals or bacteriostatics against both bacterial groups; they significantly reduced the maximum growth rate in comparison with control samples.

The response surface model have given a higher R² value and smaller RMSE which indicate a good fit of the experimental data to the model. In other words, the model describes the data precisely for both bacterial groups. However, validation may be necessary to compare between model data and experimental validation data, and to identify those circumstances where the model can be relied upon and where it could fail.

Chapter 10

10 General discussion and future work

General Discussion

Concerns over food safety and quality of perishable food in particular RTE meat product worldwide, have elevated the importance of food microbiology to an all-time high. Consequently, there is a growing need for comprehensive information about the occurrence and survival of the food-borne pathogens associated to RTE meat products, which allows better shelf life prediction.

This study aimed to evaluate whether natural spice extracts that play an important role in these foods (Figure 10.1), in relation to other hurdle techniques to improve the safety and the quality of doner kebab, and whether if they would extend the shelf-life of the product.

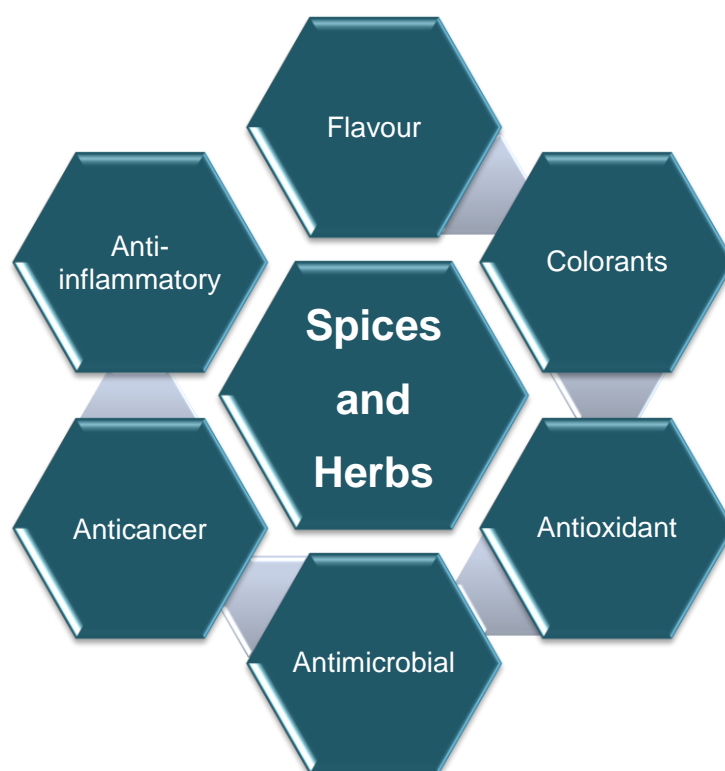


Figure 10.1: An overview of the potential of spices and aromatic herbs as functional foods.

Data in Chapter 4 showed the influence of two types of extracts (oleoresin and aquaresin) of edible plants (rosemary, cinnamon, clove, black pepper, spearmint and sumac) against ten strains of pathogenic and spoilage bacteria *in vitro* and also showed their antioxidant property in lamb fat model. All spice extracts demonstrated antimicrobial effects against pathogenic and spoilage bacteria with different efficacy; Gram-positive bacteria were more sensitive to these, compared to Gram-negative strains. Current results are in line with previous studies (Shan et al. 2007b, Weerakkody et al. 2010). It has been hypothesised by Kalemba and Kunicka (2003), that this is attributable to the differences in the cell wall structure; spice extracts can penetrate through the cell wall of Gram-positive bacterial and attack the cytoplasmic membrane, leading to not only leakage of the cytoplasm but also cytoplasm coagulation.

Interestingly, both of cinnamon oleoresin and sumac alcoholic extracts exhibited a broad spectrum of antimicrobial activity against pathogenic and spoilage bacteria, with a minimum inhibitory concentration.

An agar well diffusion method was employed to determine the diameter of inhibition zone (DIZ) and the minimum inhibitory concentration (MIC) of selected spice extracts. This technique is widely used for assaying plant extracts for their antimicrobial activity, which provided a preliminary indication of the potential activity of extracts. The specific advantages of this method represented by the possibility of testing six to eight extracts per plate against a single microorganism, with small quantities of sample required it was easily performed (Al-Kutby et al. 2009). However, the outcome of this test can be affected by many factors including the method used to extract the spice and herbs, the inoculum size, growth phase, culture medium, pH of the media and incubation time and temperature (Burt 2004).

Antioxidant activity of spice extracts in a lamb fat model was expressed as an stability index, which is an important parameter in the characterization of antioxidant of spice extracts assessed by using the Rancimat method. The method offers a real alternative for evaluation the oxidative stabilities due to the appreciable saving in labor when comparing with others such as the active oxygen method (AOM)(Läubli and Bruttel 1986).

Among all the spice extracts tested, the rosemary oleoresin was the most effective extract to stabilise the lamb fat. According to Nakatani (1997) and Carvalho et al.(2005), Rosmanol, epirosmanol, isorosmanol, and carnosol represent the antioxidant potency of rosemary extracts.

Positive linear relationships between the stability indices (SI) and spice extracts concentrations were observed for all tested spice extracts, except for sumac aqueous extracts that showed a negative correlation, indicating their pro-oxidant effect.

Those extracts that showed promising *in vitro* antioxidant and antibacterial activity were used for the next step to determine their efficacy, stability, and bioavailability in doner kebab model.

The validation of shelf-life is important for ensuring the safety and the quality of RTE cooked meat products, specifically during new product development, when shelf life studies for an existing product are absent, or after modification, or reformulation of existing products (Food Safety Authority of Ireland 2011)

One of the principal methods of predicting the rancidity-linked shelf life of RTE food is to monitor the level of lipid oxidation in high fat cooked meat products stored at elevated temperatures. Lipid oxidation is considered as one of the fundamental reactions in food chemistry, and the degree of lipid oxidation has

great consequences for both meat quality and acceptability (Verleyen et al. 2005) which is influenced by several factors (Figure 10.2).

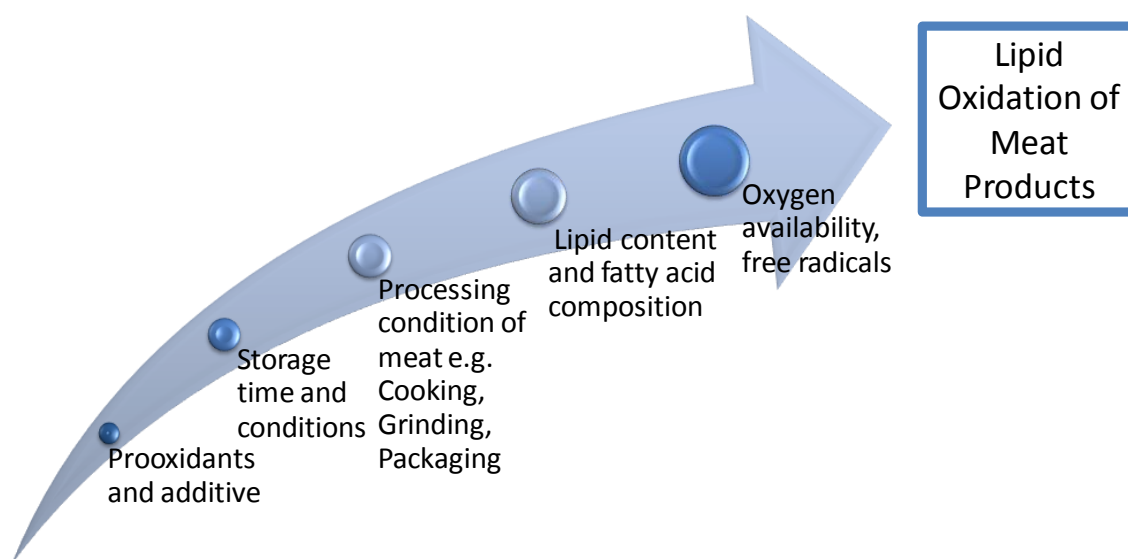


Figure 10.2: Factors influenced the rate of lipid oxidation in cooked meat products

The lipid oxidation of doner kebab samples was measured by using both TBA and the SI (Rancimat) methods. TBA values obtained by a distillation procedure appear to be more sensitive and more suitable to determine the lipid oxidation, particularly for high fat meat products. In addition, this method avoids the interference caused by pigments from natural plant sources (Ganhão et al. 2011).

In Chapter 5, both normal and accelerated shelf life studies were conducted on RTE cooked doner kebab samples. For the accelerated study, the lipid oxidation of doner kebab sample (TBAR values and SI) was linearly related with storage time. The oxidation rate of doner kebab sample increased with time and was significantly decreased at low temperature, with rosemary oleoresin (Estévez and Cava 2006, Doolaege et al. 2012) and under vacuum packaging (Viuda-Martos et al. 2010a).

On the other hand, studying the normal shelf life and the chemical composition helps the understanding of factors such as the pH and a_w , fat content, associated microflora and microbiological status of the product.

Data presented in Chapter 6 specify the influence of chilled storage, spice extracts (RO, CO, SO and SRC) and packaging conditions (VP and MAP) on the safety and quality of RTE doner kebab by studying many indices. These include changes in physico-chemical properties, bacterial levels, chemical levels (TBA), and sensory acceptability. All these indices provided an entire picture about doner kebab quality and safety.

- Microbiological test

A remarkable contribution for safety and quality assessment by bacterial population was found by monitoring the growth of total viable count and lactic acid bacteria levels of RTE doner kebab. Cinnamon (CH), rosemary (RH) and the combination of sumac, rosemary and cinnamon (SRC) extracts significantly reduced TVC for both VP and MAP pack when comparing with control and sumac treated samples. At the end of the experiment, CH and SRC samples both reduced the final bacterial count for about 2 log cycle compared with control, SH, and SL; and 1 log cycle when compared with RH, RL, and CL. All spice extracts act as a bactericidal against LAB with different significance, higher effects were obtained from CH samples and under VP storage.

- Sensory acceptability

Sensory evaluation is an important step for new product development that measured both consumer preference (Guàrdia et al. 2006) and the shelf life of the product (Stolzenbach et al. 2009). In general, the ranking test showed that a consumer panel had preference towards spice extracts addition in doner kebab samples for the acceptability of colour, aroma, overall acceptance and overall appearance scores. The Ranking test is a rapid method applied to compare

several samples for a single attribute, component of attribute or total impression (British Standards 5929 1986b).

For the shelf life determination, a hedonic test by a selected panel was used to measure the effect of spice extracts and packaging on product acceptability during the shelf life at 4°C. The results confirmed that the sensory scores are time- dependent, as they decreased as storage time progressed. Out of the five attributes tested, only the aroma and acceptability scores provided useful information about the effect of spice extract addition on doner kebab sample.

All these parameters mentioned above, help to identify the hurdle technology for effective shelf life extension to obtain safe and good quality RTE doner kebab (Figure 10.3)

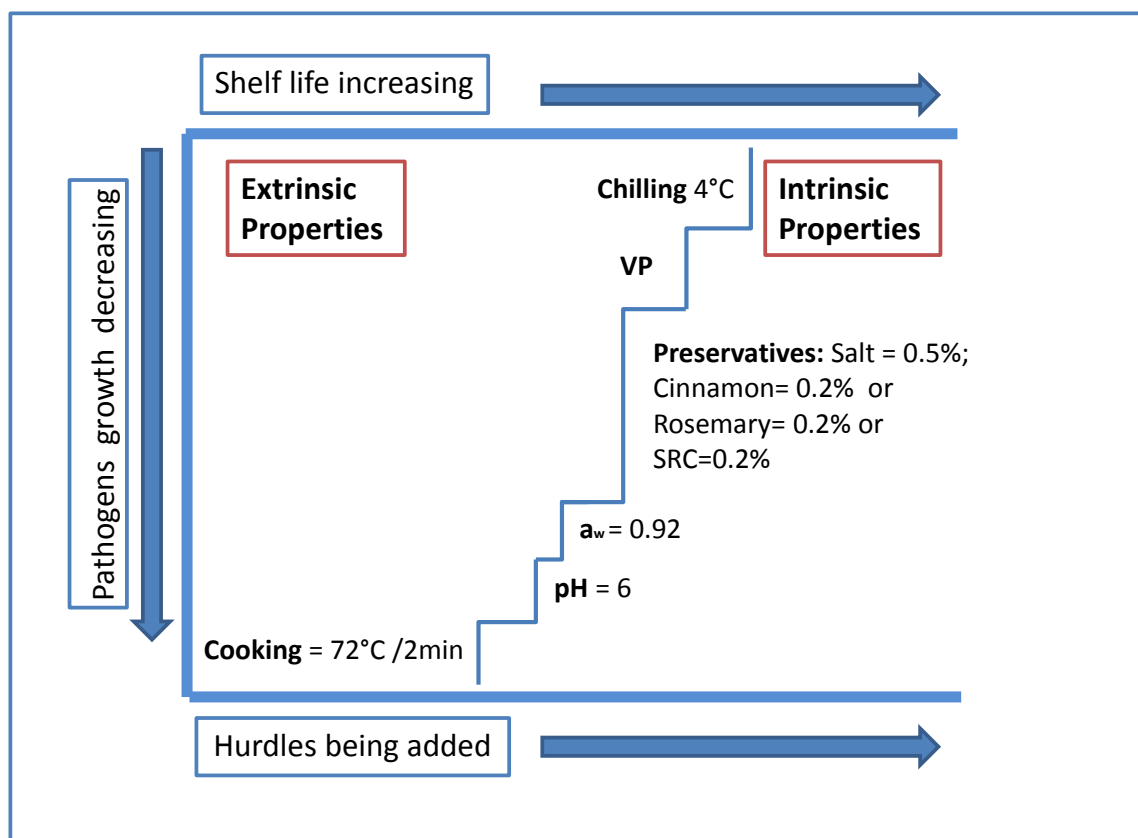


Figure 10.3: Hurdle technology in RTE cooked doner kebab

Physical, chemical and microbiological tests for assessing the shelf life of food are suitable for research and product development; however, they are not practical for routine use, since they need trained staff and expensive laboratory equipment, and they can be both time consuming and labour intensive. Thus, a fast reliable test is preferable to guarantee food safety and quality of meat products during the storage time.

Challenge tests are a laboratory-based studies which aim to determine the behaviour and the ability of food-borne pathogens to grow in artificially contaminated foods as well as to identify how fast they will grow (Beaufort et al. 2008). According to the Food Safety Authority of Ireland (2011), such test is required for food products to:

- Evaluate potential risks for the survival, growth and/or toxins production by food-borne pathogens
- Describe the important controlling factors, e.g. pH, a_w , storage temperature, for specific pathogens that have not been defined by the food business
- When no safety data are available

Post-process contamination associated with *L. monocytogenes* in ready-to-eat meat products possess a real threat for public health (Jaroni et al. 2010). Thus, in Chapter 7, challenge test was carried out to determine the behaviour of *L. monocytogenes* in MAP refrigerated RTE doner kebab control and samples treated with rosemary, cinnamon, sumac and their combination (SRC). These were applied to the products at two different concentrations either before cooking (BC) or after cooking (AC). Treatments were evaluated for their effect on the growth of *L. monocytogenes*, TVC and LAB during storage time. The results obtained in this study highlighted the important role of spice extracts for controlling the growth of *L. monocytogenes*, TVC and LAB with various degrees of antimicrobial efficiency. Interestingly, when spice extracts were applied after

cooking (AC), they demonstrated a stronger antimicrobial effect although lower concentration was used (Al-Kutby et al. 2010) . The likely explanation of that is summarised in Figure 10.4

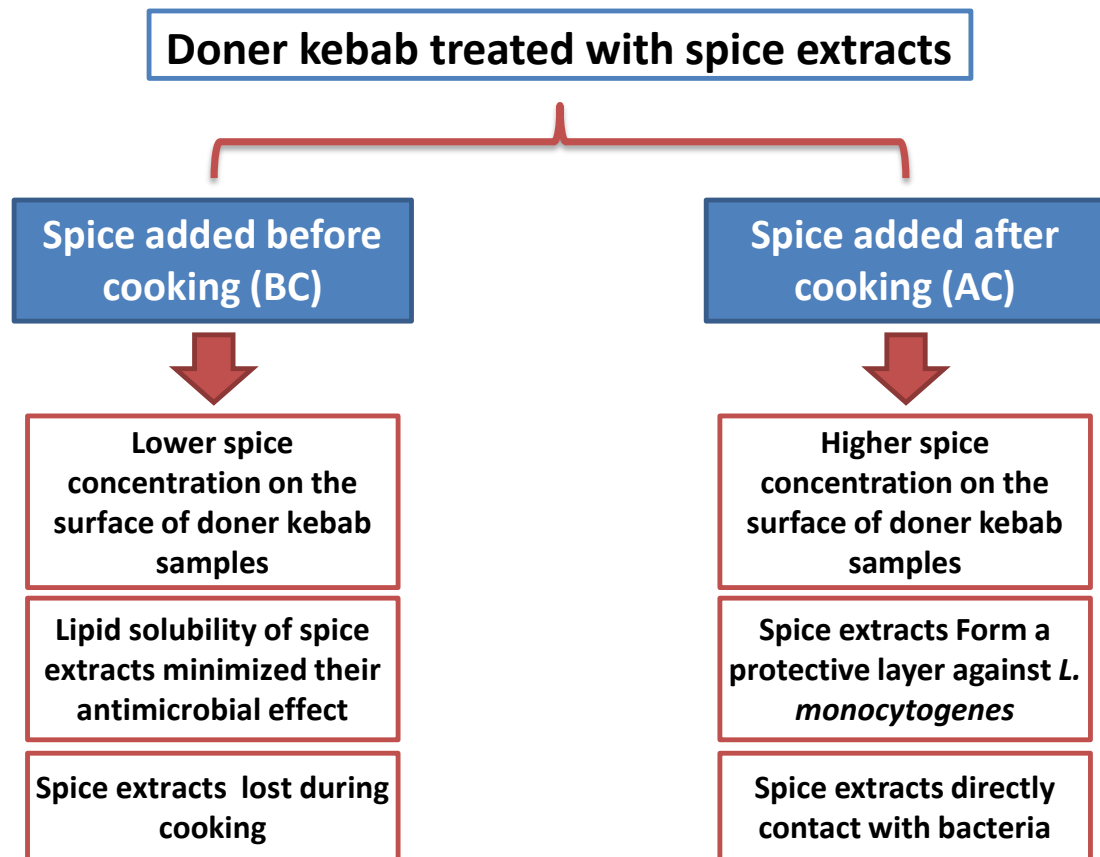


Figure 10.4: Application methods and spice effectiveness factors.

A challenge testing protocol is useful for assessing the behaviour of *L. monocytogenes* in RTE meat products and can provide useful data for determining the safety and shelf life of RTE food under a set of conditions. However, this method must always be used with caution. It is strongly recommended that researchers only perform a challenge testing protocol if they have proper facilities and a good understanding of these microbiological techniques which includes evaluation of product characteristics, shelf-life of the product, number of batches, choice of the strain, preparation of the inoculum, storage conditions, measurement of physical-chemical characteristics, and microbiological analyses (Beaufort et al. 2008).

The time-temperature relationships related to the cooking and cooling process of doner kebab are considered as a main concern from a public health standpoint. Based on both the cooking practices and the big size of doner kebab pieces which allow variations in the temperature control of the product, the growth of spore forming bacteria in general and *C. perfringens* in particular is possible.

Therefore, Chapter 8 study focused efforts to resolve spore-forming bacteria in RTE doner kebab samples during cooking and cooling time. The results showed that both the thermal inactivation (D-value) and the thermal resistance (z-value) for vegetative cell of *C. perfringens* and *B. cereus* followed first order kinetics and were significantly reduced by the application of sumac extracts (Al-Kutby et al. 2011). Obtained D and Z values can be used by the food industry to predict the thermal destruction time of spore forming bacteria. Judging from the results from this experiment, it is important to advice food possessors that doner kebab could be reformulated by adding sumac extract as an ingredient to reduce the risk associated with spore forming bacteria.

The target in applying HACCP principles in meat products is to have the operator take intensive actions to ensure safe food. The effective and practical method of hazard control exemplifies the concept of active managerial control by providing an on-going system of simple control measures that will reduce the occurrence of risk factors that lead to out-of-control hazards (Food and Drug Administration 2005). Therefore, in order to characterize the risk and identify the critical control point related to spore forming bacteria in RTE doner kebab, time-temperature profiles during both cooking and cooling periods were determined (Chapter 8). This would facilitate the optimization of both processes, considering process lethality, log reduction and growth prediction of *C.*

perfringens. This type of information will bring benefits towards the performance standard regarding the safety of RTE doner kebab. Thus, the growth of *C. perfringens* was predicted by using both cooling profile and pathogen modelling program (PMP)/ Cooling growth model. The specific models are commonly used, freely available and developed to predict the behaviour of *C. perfringens* during the cooling time in meat product to provide a reasonable estimation for growth. However, accurate information will be provided only when trained microbiologists with appropriate skills and experience interpret the model.

In addition, the validation of the result by experimental inoculation of *C. perfringens* spores in doner kebab samples (challenge test) will help to obtain actual data to derive more precise results than PMP to determine safe cooling regimes. Such exercise was left out of the scope of this project, due to both time and resource limitations.

Based on (Health Protection Agency 2009c) advice, predicted microbiology in RTE cooked meat products such as doner kebab in the UK could contribute to:

- Reduce the risk associated with RTE meat product during production, holding, distribution and storage condition
- Determine the relative shelf life depending on the storage conditions
- Develop products (reformulation, minor process change) to meet the Regulation on microbiological criteria for RTE food e.g. Food Standards Agency, UK

In Chapter 9, primary and secondary models were used to predict the growth of pathogenic bacteria related to RTE doner kebab represented by both *L. monocytogenes* and *S. Typhimurium* under different conditions. The Baranyi and Roberts model have given accurate and reliable estimates of growth kinetics parameters for both bacterial groups. Thus, the use of this model to predict the growth rate for subsequent secondary modelling (RSA) is recommended. Both models indicated that the growth rate of *L. monocytogenes*

and *S. Typhimurium* were dependent on the interaction of the four tested variables (temperature, pH, NaCl, and spice extracts). The results suggest that the tested spice extracts (cinnamon, rosemary and sumac) have significant bacteriostatic and/or bactericidal activity against *L. monocytogenes* and *S. Typhimurium*, and may offer RTE meat products a degree of protection against these pathogens, especially if employed in combination with acidic pH, high NaCl concentrations, and adequate refrigeration.

Predictive mathematical models can offer a cost-effective means to minimise microbial testing in predicting the behaviour of pathogens under different conditions and to determine the shelf life of the products. However, there may be occasions when the models predictions may not be precise (Food Safety Authority of Ireland 2011). Therefore, the applicability of model to predict the growth of pathogenic bacteria in food must be checked cautiously before prediction is used in commercial circumstances due to inconsistent microbial responses, and variations in the growth media and growth conditions (Food Safety Authority of Ireland 2011).

Such checking could be done either by comparison with independent data in the literature or by validation of the model.

The bacterial growth curves were determined by using a plate reader method, which is based on the turbidity measurements which provided rapid results and contributed to time and resources saving when compared to the conventional microbiological total plate count methods that require more expensive laboratory equipment and staff training. Indeed, many studies reported that turbidity measurement data could be used for model development to estimate the growth rate of microorganisms instead of viable count measurements (Dalgaard and Mejlholm 1997, Nerbrink et al. 1999)

In this study, however the plate reader was suitable only for taking readings at ambient temperature and above due to the issue of condensation on the microplate lids at chilled temperatures. This condensation interfered with readings and subsequently generated unreliable absorbance data which needed careful checking (for example the growth curve of *L. monocytogenes* at 4°C for all conditions tested were excluded from the results in Chapter 9). The other drawbacks were that it measure both live and dead cells, and that it is limited by high detection thresholds, thus these techniques are restricted to conditions where high cell densities are reached such as spoilage bacteria in foods (Dalgaard and Koutsoumanis 2001).

Future work

There is continued need to:

- Develop and validate the microbial mathematical models for growth, survival and death of pathogenic and spoilage bacteria in food business operators and food processing establishments where there is exposure to different environmental conditions. This will help to minimise the risk associated with these microorganisms in doner kebab during cooking, cooling, storage, distribution, and adding decision to current control strategies.
- The practice of shaving the product surface during retail has not been studied; the safe depth of the doner kebab slice and time –temperature profiles during cooking need further investigation and validation.
- The action of sumac extracts components on proteins embedded in the cytoplasmic membrane and on phospholipids in the membrane is not yet fully identified and it could be a focal area for future research. Further

elucidation of these mechanisms against pathogenic and spoilage bacteria related to doner kebab is important and would provide insights that may prove useful for technological applications. The use of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analysis to examine the mechanisms of action of extracts against cell membranes (Fei et al. 2011) and to further analyse the morphological abnormalities in the bacterial strains (Hu et al. 2011).

- The application of sumac in RTE doner kebab products considerably reduces the decimal reduction time D-value, thermal resistance Z-value and the log reduction for the vegetative cell of *C. perfringens* and *B. cereus*. Thus, it might be relevant to investigate the effect of sumac extract on the spores of these bacteria in RTE doner kebab.
- Interactions between sumac extracts and their components and these with other food ingredients and food additives need further investigation.
- Possible secondary consequences of the use of sumac extract would need to be investigated. Whether if the addition of sumac has any disadvantageous effect on the safety of the RTE meat products, for example influencing the stress tolerance of pathogens.

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Appendix A

Supplementary data (Figures and Tables)

A.1 Data relevant for chapter (4)

Stability index (SI) of spice extracts depending on the mean of different concentrations.

| Spice extracts | Means \pm SE of Stability index * | Regression | | |
|----------------|-------------------------------------|------------|------|------|
| | | a** | b** | r** |
| RO | 14.75 \pm 2.44 ^a | 8.41 | 7.18 | 0.92 |
| RA | 5.10 \pm 0.97 ^b | 3.69 | 1.78 | 0.97 |
| BPO | 3.50 \pm 0.68 ^{bc} | 2.75 | 1.03 | 0.98 |
| SO | 3.03 \pm 0.54 ^{bc} | 2.51 | 0.77 | 0.99 |
| BPA | 2.84 \pm 0.53 ^{bc} | 2.46 | 0.63 | 0.99 |
| CLA | 1.83 \pm 0.21 ^{bc} | 0.91 | 1.01 | 0.98 |
| CLO | 1.53 \pm 0.18 ^{bc} | 0.81 | 0.81 | 0.97 |
| CO | 1.49 \pm 0.12 ^{bc} | 0.56 | 0.99 | 0.99 |
| CA | 1.31 \pm 0.09 ^{bc} | 0.42 | 0.94 | 0.98 |
| MA | 0.92 \pm 0.03 ^d | 0.05 | 0.88 | 0.97 |
| SA | 0.79 \pm 0.06 ^d | - 0.29 | 1.06 | 0.96 |
| Control | 1 \pm 0.00 ^d | | | |

* Stability index = induction time (conductivity curves) of the sample/ induction time of the control; ^{a-d} Means within a column with different letters are significantly different (P < 0.05); a**: intercept, b**: coefficient (stability index/concentration), r**: correlation coefficient, Key to the table is listed under Table 4.1.

A. 2 Data relevant for chapter (6)

Lactic acid bacteria (LAB) counts (log CFUg⁻¹) in refrigerated vacuum pack ready to eat doner kebab with added spice extracts, during storage time.

| Time (weeks) | C | SRC | SH | SL | RH | RL | CH | CL |
|--------------|---------------------|--------------------|--------------------|----------------------|---------------------|---------------------|--------------------|--------------------|
| 0 | 3.60 ^{ax} | 2.47 ^{ay} | 3.34 ^{aw} | 3.44 ^{ax} | 3.20 ^{aw} | 3.54 ^{ax} | 1.80 ^{az} | 2.20 ^{av} |
| 2 | 3.41 ^{bx} | 2.15 ^{by} | 2.94 ^{bw} | 3.28 ^{abxv} | 3.15 ^{awv} | 3.36 ^{axv} | n.d. ^{bz} | 2.15 ^{ay} |
| 4 | 3.47 ^{bdx} | n.d. ^{cy} | 2.19 ^{cz} | 3.17 ^{bcx} | n.d. ^{by} | n.d. ^{by} | n.d. ^{by} | n.d. ^{by} |
| 6 | 3.56 ^{adx} | n.d. ^{cy} | n.d. ^{dy} | 3.09 ^{cz} | n.d. ^{by} | n.d. ^{by} | n.d. ^{by} | n.d. ^{by} |
| 8 | 3.65 ^{ax} | n.d. ^{cy} | n.d. ^{dy} | n.d. ^{dy} | n.d. ^{by} | n.d. ^{by} | n.d. ^{by} | n.d. ^{by} |
| 10 | 3.77 ^{acx} | n.d. ^{cy} | n.d. ^{dy} | n.d. ^{dy} | n.d. ^{by} | n.d. ^{by} | n.d. ^{by} | n.d. ^{by} |
| 12 | 3.86 ^{cx} | n.d. ^{cy} | n.d. ^{dy} | n.d. ^{dy} | n.d. ^{by} | n.d. ^{by} | n.d. ^{by} | n.d. ^{by} |

For sample denomination see Figure 6.1.

n.d.: not detected (<1.69; detection limit).

^{a-d} Means within a column with different letters are significantly different ($P < 0.05$).

^{v-z} Means within a row with different letters are significantly different ($P < 0.05$)

LSD at 5%= 0.238

Lactic acid bacteria (LAB) counts (log CFUml⁻¹) in refrigerated modified atmosphere pack ready to eat doner kebab with added spice extracts, during storage time.

| Time (weeks) | C | SRC | SH | SL | RH | RL | CH | CL |
|--------------|---------------------|--------------------|---------------------|---------------------|----------------------|---------------------|---------------------|----------------------|
| 0 | 3.30 ^{ax} | 3.12 ^{ax} | 3.24 ^{ax} | 3.30 ^{ax} | 3.14 ^{ax} | 3.23 ^{ax} | 2.77 ^{ay} | 2.83 ^{acy} |
| 2 | 2.84 ^{bxv} | 2.39 ^{by} | 2.87 ^{bxw} | 2.85 ^{bxw} | 2.95 ^{abxw} | 3.15 ^{aw} | 2.54 ^{avy} | 2.87 ^{axw} |
| 4 | 3.00 ^{bx} | 1.96 ^{cy} | 2.27 ^{cw} | 2.69 ^{bfz} | 2.85 ^{bxz} | 2.84 ^{bxz} | 2.06 ^{by} | 2.78 ^{acxz} |
| 6 | 3.46 ^{ax} | n.d. ^{dy} | 2.17 ^{cew} | 2.63 ^{bfv} | 2.38 ^{cw} | 2.59 ^{bcv} | n.d. ^{cy} | 2.55 ^{bcv} |
| 8 | 3.42 ^{ax} | n.d. ^{dy} | 2.06 ^{cez} | 2.50 ^{cfw} | 2.35 ^{cw} | 2.49 ^{cw} | n.d. ^{cy} | 2.31 ^{bw} |
| 10 | 3.39 ^{ax} | n.d. ^{dy} | 2.00 ^{dez} | 2.16 ^{dz} | 1.84 ^{dy} | 2.03 ^{dz} | n.d. ^{cy} | 1.89 ^{dy} |
| 12 | 3.40 ^{ax} | n.d. ^{dy} | n.d. ^{fy} | n.d. ^{ey} | n.d. ^{ey} | n.d. ^{ey} | n.d. ^{cy} | n.d. ^{ey} |

For sample denomination see Figure 6.1.

n.d.: not detected (<1.69; detection limit).

^{a-d} Means within a column with different letters are significantly different ($P < 0.05$).

^{w-z} Means within a row with different letters are significantly different ($P < 0.05$).

LSD at 5%= 0.265

TBA values of VP doner kebab samples during storage time 4°C.

| Treatments | Time (weeks) | | | | | | |
|------------|--------------------------|---------------------------|-------------------------|---------------------------|---------------------------|-------------------------|-------------------------|
| | 0 | 2 | 4 | 6 | 8 | 10 | 12 |
| C | 0.47±0.01 ^{awy} | 0.50±0.01 ^{abwy} | 0.51±0.00 ^{bw} | 0.59±0.00 ^{cwy} | 0.64±0.00 ^{dw} | 0.72±0.01 ^{ew} | 0.78±0.00 ^{fw} |
| CH | 0.35±0.01 ^{aw} | 0.41±0.01 ^{bw} | 0.46±0.03 ^{bw} | 0.55±0.01 ^{cw} | 0.60±0.00 ^{cw} | 0.62±0.02 ^{cw} | 0.62±0.00 ^{cw} |
| CL | 0.40±0.01 ^{awy} | 0.44±0.01 ^{awy} | 0.50±0.01 ^{bw} | 0.59±0.01 ^{cwy} | 0.63±0.01 ^{cwy} | 0.70±0.00 ^{dw} | 0.74±0.02 ^{dw} |
| SRC | 0.38±0.02 ^{aw} | 0.43±0.00 ^{abw} | 0.45±0.01 ^{bw} | 0.55±0.01 ^{cw} | 0.54±0.02 ^{cw} | 0.62±0.00 ^{dw} | 0.63±0.02 ^{dw} |
| RH | 0.34±0.00 ^{aw} | 0.37±0.01 ^{abw} | 0.43±0.01 ^{bw} | 0.51±0.01 ^{cwyw} | 0.56±0.02 ^{dw} | 0.59±0.02 ^{ew} | 0.61±0.00 ^{dw} |
| RL | 0.43±0.00 ^{awy} | 0.44±0.01 ^{awy} | 0.49±0.00 ^{bw} | 0.62±0.00 ^{cwy} | 0.66±0.00 ^{cdwy} | 0.66±0.02 ^{dw} | 0.71±0.00 ^{ew} |
| SH | 0.47±0.00 ^{az} | 0.49±0.00 ^{az} | 0.57±0.00 ^{bz} | 0.68±0.01 ^{cz} | 0.76±0.01 ^{dz} | 0.82±0.00 ^{ez} | 0.86±0.01 ^{fz} |
| SL | 0.48±0.01 ^{az} | 0.49±0.00 ^{az} | 0.57±0.01 ^{bz} | 0.66±0.01 ^{cz} | 0.77±0.01 ^{dz} | 0.83±0.00 ^{ez} | 0.88±0.01 ^{fz} |

Values are given as mean ± SE, ^{a-f} mean in the same columns with different letter are significantly difference ($P < 0.05$), ^{w-z} mean in the same rows with different letter are significantly difference ($P < 0.05$) For sample key see Figure 6.1

TBA values of MAP doner kebab samples during storage time at 4°C.

| Treatments | Time (weeks) | | | | | | |
|------------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|--------------------------|--------------------------|
| | 0 | 2 | 4 | 6 | 8 | 10 | 12 |
| C | 0.48±0.03 ^{ay} | 0.60±0.01 ^{by} | 0.71±0.01 ^{cy} | 0.77±0.02 ^{cdy} | 0.82±0.02 ^{dey} | 0.88±0.02 ^{ey} | 0.98±0.01 ^{fy} |
| CH | 0.36±0.01 ^{ax} | 0.56±0.02 ^{bx} | 0.59±0.01 ^{bx} | 0.64±0.02 ^{cx} | 0.66±0.01 ^{cdx} | 0.67±0.01 ^{dx} | 0.69±0.01 ^{dx} |
| CL | 0.42±0.01 ^{axy} | 0.59±0.01 ^{bcxy} | 0.68±0.01 ^{bcxy} | 0.68±0.01 ^{cdxy} | 0.73±0.03 ^{cdxy} | 0.75±0.01 ^{dxy} | 0.80±0.01 ^{dxy} |
| SRC | 0.37±0.02 ^{ax} | 0.53±0.01 ^{bx} | 0.58±0.02 ^{bx} | 0.64±0.00 ^{cx} | 0.67±0.02 ^{cdx} | 0.69±0.01 ^{cdx} | 0.72±0.02 ^{dx} |
| RH | 0.37±0.01 ^{ax} | 0.57±0.01 ^{bx} | 0.59±0.02 ^{bcx} | 0.60±0.03 ^{bcx} | 0.65±0.01 ^{cdx} | 0.68±0.01 ^{dx} | 0.72±0.03 ^{dx} |
| RL | 0.38±0.02 ^{axy} | 0.59±0.01 ^{bxy} | 0.64±0.01 ^{cxy} | 0.71±0.01 ^{dxy} | 0.76±0.01 ^{dxy} | 0.81±0.01 ^{exy} | 0.86±0.02 ^{fx} |
| SH | 0.40±0.01 ^{az} | 0.70±0.02 ^{bz} | 0.79±0.01 ^{cz} | 0.83±0.01 ^{dz} | 1.03±0.01 ^{ez} | 1.18±0.03 ^{fz} | 1.29±0.01 ^{gz} |
| SL | 0.39±0.01 ^{az} | 0.73±0.01 ^{bz} | 0.81±0.02 ^{cz} | 0.86±0.01 ^{dz} | 0.99±0.02 ^{ez} | 1.17±0.01 ^{fz} | 1.28±0.01 ^{gz} |

Values are given as mean ± SE, ^{a-f} mean in the same columns with different letter are significantly difference ($P < 0.05$), ^{x-z} mean in the same rows with different letter are significantly difference ($P < 0.05$). For sample key, see Figure 6.1.

Evaluation of Aroma score of doner kebab samples for different treatment conditions, over storage time at refrigerated temperature.

| Treatments | | Time (weeks) | | | | | | |
|------------|-----|--------------------------|--------------------------|--------------------------|---------------------------|---------------------------|--------------------------|--------------------------|
| | | 0 | 2 | 4 | 6 | 8 | 10 | 12 |
| VP | CH | 4.7 ± 0.6 ^{awx} | 4.2 ± 0.4 ^{aw} | 4.3 ± 0.7 ^{aw} | 3.9 ± 0.4 ^{aw} | 3.6 ± 0.3 ^{aw} | 3.5 ± 0.3 ^{aw} | 3.4 ± 0.2 ^{aw} |
| | CL | 5.0 ± 0.7 ^{awx} | 4.7 ± 0.3 ^{aw} | 5.0 ± 0.7 ^{aw} | 5.0 ± 0.6 ^{aw} | 4.3 ± 0.6 ^{aw} | 4.0 ± 0.3 ^{aw} | 3.7 ± 0.6 ^{aw} |
| | SRC | 4.3 ± 0.5 ^{aw} | 4.2 ± 0.5 ^{aw} | 4.0 ± 0.6 ^{aw} | 4.3 ± 0.6 ^{aw} | 4.0 ± 0.6 ^{aw} | 3.8 ± 0.3 ^{aw} | 3.7 ± 0.2 ^{aw} |
| | C | 5.7 ± 0.3 ^{awx} | 5.2 ± 0.5 ^{aw} | 5.0 ± 0.5 ^{abw} | 4.8 ± 0.5 ^{abcw} | 4.7 ± 0.3 ^{abcw} | 3.8 ± 0.4 ^{bcw} | 3.7 ± 0.2 ^{cw} |
| | RH | 5.3 ± 0.7 ^{awx} | 5.2 ± 0.6 ^{aw} | 5.2 ± 0.5 ^{aw} | 4.8 ± 0.6 ^{abw} | 4.2 ± 0.6 ^{abw} | 4.8 ± 0.4 ^{abw} | 3.7 ± 0.8 ^{bw} |
| | RL | 6.0 ± 0.6 ^{ax} | 5.3 ± 0.6 ^{aw} | 5.3 ± 0.3 ^{aw} | 5.0 ± 0.5 ^{abw} | 4.7 ± 0.7 ^{abw} | 4.7 ± 0.2 ^{abw} | 3.7 ± 0.3 ^{bw} |
| | SH | 5.5 ± 0.6 ^{awx} | 5.0 ± 0.5 ^{abw} | 4.8 ± 0.5 ^{abw} | 5.0 ± 0.6 ^{abw} | 4.3 ± 0.2 ^{bw} | 4.3 ± 0.2 ^{bw} | 3.3 ± 0.4 ^{cw} |
| | SL | 5.7 ± 0.6 ^{awx} | 5.3 ± 0.5 ^{aw} | 5.2 ± 0.3 ^{abw} | 4.8 ± 0.4 ^{abw} | 4.0 ± 0.4 ^{bcw} | 4.7 ± 0.4 ^{abw} | 3.3 ± 0.6 ^{cw} |
| MAP | CH | 4.8 ± 0.9 ^{aw} | 4.2 ± 0.7 ^{awx} | 3.8 ± 0.8 ^{abw} | 3.5 ± 0.7 ^{abw} | 4.0 ± 0.8 ^{aw} | 3.3 ± 0.6 ^{aw} | 3.3 ± 0.6 ^{aw} |
| | CL | 4.7 ± 0.6 ^{awx} | 4.7 ± 0.8 ^{ax} | 5.0 ± 0.2 ^{awx} | 4.3 ± 0.4 ^{abw} | 4.2 ± 0.4 ^{aw} | 4.0 ± 0.3 ^{awx} | 4.0 ± 0.5 ^{awx} |
| | SRC | 4.7 ± 0.8 ^{aw} | 4.5 ± 0.8 ^{awx} | 4.2 ± 0.5 ^{aw} | 4.0 ± 0.4 ^{aw} | 4.2 ± 0.7 ^{aw} | 4.3 ± 0.4 ^{awx} | 3.7 ± 0.3 ^{awx} |
| | C | 5.8 ± 0.7 ^{ax} | 4.8 ± 0.4 ^{awx} | 5.2 ± 0.6 ^{awx} | 4.8 ± 0.5 ^{aw} | 4.7 ± 0.6 ^{aw} | 4.3 ± 0.3 ^{awx} | 3.8 ± 0.6 ^{bw} |
| | RH | 5.3 ± 0.7 ^{awx} | 5.2 ± 0.3 ^{awx} | 4.5 ± 0.9 ^{aw} | 4.8 ± 0.6 ^{aw} | 4.8 ± 0.5 ^{aw} | 4.8 ± 0.5 ^{ax} | 4.0 ± 0.7 ^{awx} |
| | RL | 5.3 ± 0.6 ^{awx} | 5.2 ± 0.4 ^{awx} | 4.8 ± 0.2 ^{abx} | 4.8 ± 0.3 ^{abw} | 4.8 ± 0.5 ^{abw} | 5.0 ± 0.6 ^{abx} | 3.8 ± 0.5 ^{bw} |
| | SH | 5.8 ± 0.7 ^{ax} | 4.8 ± 0.7 ^{abw} | 5.2 ± 0.4 ^{abw} | 4.3 ± 0.3 ^{bw} | 5.0 ± 0.4 ^{abw} | 4.2 ± 0.5 ^{bw} | 4.2 ± 0.5 ^{bw} |
| | SL | 5.3 ± 0.7 ^{awx} | 4.9 ± 0.6 ^{awx} | 5.1 ± 0.2 ^{ax} | 4.8 ± 0.5 ^{aw} | 5.0 ± 0.5 ^{aw} | 4.7 ± 0.4 ^{awx} | 4.4 ± 0.6 ^{ax} |

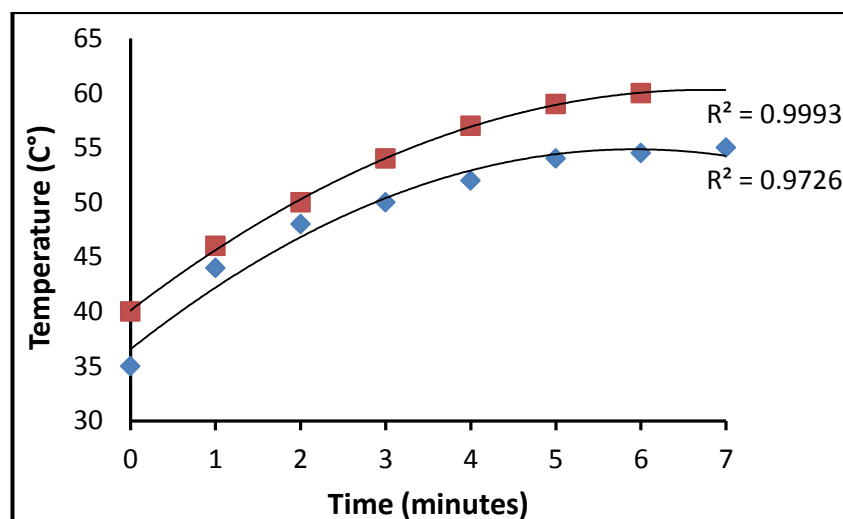
Values are given as means of six readings ± SE, ^{a-b} means followed by different letters within a row differ significantly ($P < 0.05$), ^{w-x} means followed by different letters within a column differ significantly ($P < 0.05$)

Evaluation of overall acceptance scores of doner kebab samples for different treatment conditions, over storage time at refrigerated temperature.

| Treatments | | Time (weeks) | | | | | | |
|------------|-----|-----------------------------------|------------------------|------------------------------------|-------------------------------------|-------------------------|------------------------------------|------------------------|
| | | 0 | 2 | 4 | 6 | 8 | 10 | 12 |
| VP | CH | 5.2± 0.2 ^{aw} | 4.5±0.4 ^{aw} | 4.0±0.4 ^{abw} | 3.5±0.4 ^{bw} | 4.0±0.3 ^{abw} | 3.8±0.5 ^{bw^x} | 3.2±0.3 ^{bw} |
| | CL | 5.5± 0.2 ^{aw} | 4.2±0.4 ^{abw} | 5.0±0.5 ^{aw^x} | 4.8±0.5 ^{ax} | 4.2±0.6 ^{abw} | 3.8±0.2 ^{abw^x} | 3.3±0.3 ^{bw} |
| | SRC | 5.5±0.2 ^{aw} | 4.3±0.2 ^{abw} | 4.2±0.5 ^{abw^x} | 4.5±0.3 ^{abw^x} | 4.2±0.4 ^{abw} | 4.0±0.4 ^{abw^x} | 3.3±0.3 ^{bw} |
| | C | 5.3±0.3 ^{aw} | 4.8±0.4 ^{aw} | 4.7±0.6 ^{abw^x} | 4.7±0.4 ^{abcw^x} | 4.2±0.5 ^{abcw} | 3.5±0.4 ^{bc^x} | 2.8±0.2 ^{cw} |
| | RH | 5.7±0.1 ^{aw} | 4.5±0.5 ^{abw} | 5.2±0.5 ^{aw^x} | 4.7±0.6 ^{abw^x} | 3.8±0.3 ^{bw} | 4.8±0.5 ^{aw} | 3.5±0.5 ^{bw} |
| | RL | 60±0.4 ^{aw} | 5.3±0.6 ^{aw} | 5.3±0.5 ^{ax} | 4.8±0.4 ^{ax} | 4.0±0.4 ^{bw} | 4.5±0.4 ^{abw^x} | 3.5±0.4 ^{bw} |
| | SH | 5.5±0.2 ^{aw} | 5.2±0.4 ^{aw} | 5.0±0.5 ^{aw^x} | 5.3±0.5 ^{ax} | 4.5±0.3 ^{abw} | 4.3±0.3 ^{abw^x} | 3.3±0.4 ^{bw} |
| | SL | 5.7±0.4 ^{aw} | 5.0±0.5 ^{aw} | 5.0±0.4 ^{aw^x} | 5.5±0.2 ^{ax} | 4.0±0.3 ^{abw} | 4.0±0.4 ^{abw^x} | 3.5±0.3 ^{bw} |
| MAP | CH | 5.0±0.4 ^{abw} | 5.5±0.6 ^{aw} | 4.8±0.3 ^{abw} | 4.0±0.4 ^{bw} | 4.3±0.5 ^{abw} | 4.0±0.4 ^{bw} | 3.5±0.7 ^{bw} |
| | CL | 5.8±0.2 ^{aw^x} | 4.5±0.6 ^{aw} | 5.5±0.2 ^{aw} | 4.5±0.4 ^{aw} | 4.2±0.4 ^{aw} | 4.5±0.4 ^{aw} | 4.3±0.7 ^{aw} |
| | SRC | 5.0±0.4 ^{aw^x} | 4.5±0.7 ^{aw} | 5.3±0.3 ^{bw} | 4.0±0.4 ^{aw} | 4.7±0.4 ^{aw} | 4.5±0.3 ^{aw} | 3.8±0.3 ^{aw} |
| | C | 5.8±0.3 ^{ax} | 5.0±0.4 ^{abw} | 5.0±0.5 ^{abw} | 4.5±0.4 ^{aw} | 4.7±0.5 ^{abw} | 4.7±0.3 ^{abw} | 4.0±0.5 ^{bw} |
| | RH | 5.5±0.3 ^{aw^x} | 5.7±0.4 ^{aw} | 4.7±0.6 ^{aw} | 4.3±0.4 ^{abw} | 4.8±0.3 ^{abw} | 4.5±0.4 ^{abw} | 3.8±0.7 ^{bw} |
| | RL | 5.5±0.2 ^{aw^x} | 5.3±0.5 ^{aw} | 5.5±0.2 ^{aw} | 4.7±0.4 ^{aw} | 4.7±0.3 ^{aw} | 4.3±0.4 ^{aw} | 4.5±0.5 ^{aw} |
| | SH | 6.0±0.3 ^{ax} | 5.3±0.6 ^{abw} | 5.3±0.3 ^{aw} | 4.3±0.3 ^{abw} | 5.0±0.3 ^{abw} | 4.3±0.3 ^{bw} | 3.8±0.3 ^{bw} |
| | SL | 5.8±0.5 ^{ax} | 5.2±0.5 ^{aw} | 5.3±0.2 ^{aw} | 4.7±0.4 ^{aw} | 4.8±0.3 ^{abw} | 4.3±0.4 ^{bw} | 4.7±0.6 ^{abw} |

Values are given as means of six readings ± SE, ^{a-b} means followed by different letters within a row differ significantly ($P < 0.05$), ^{w-x} means followed by different letters within a column differ significantly ($P < 0.05$), LSD at 5%=1.25, at 1%=1.64

A.3 Data relevant to chapter (8)



The time needed the broth to reach the suitable temperature in a water-bath at 55 and 60°C.

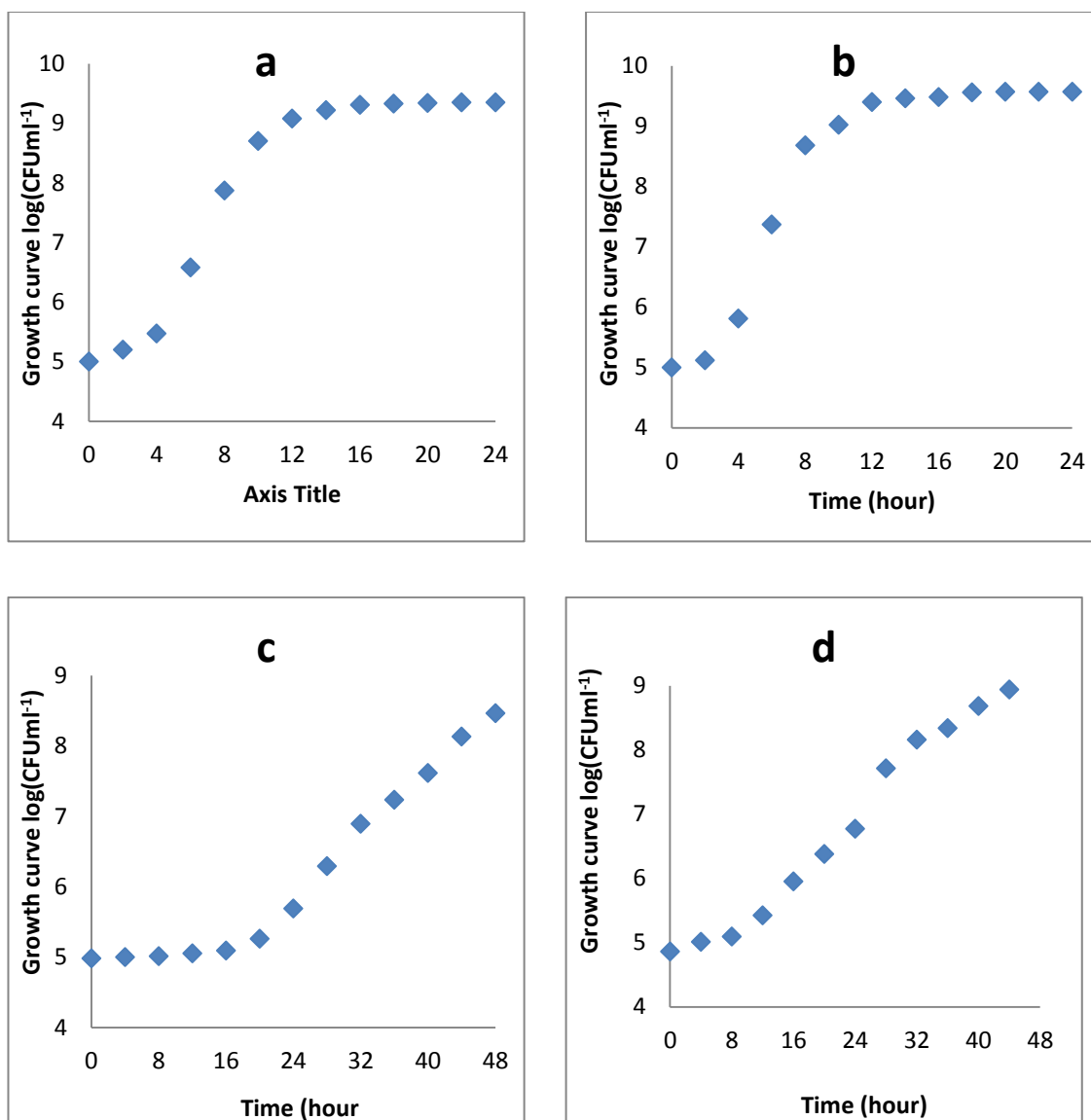


The cooking process of doner kebab.

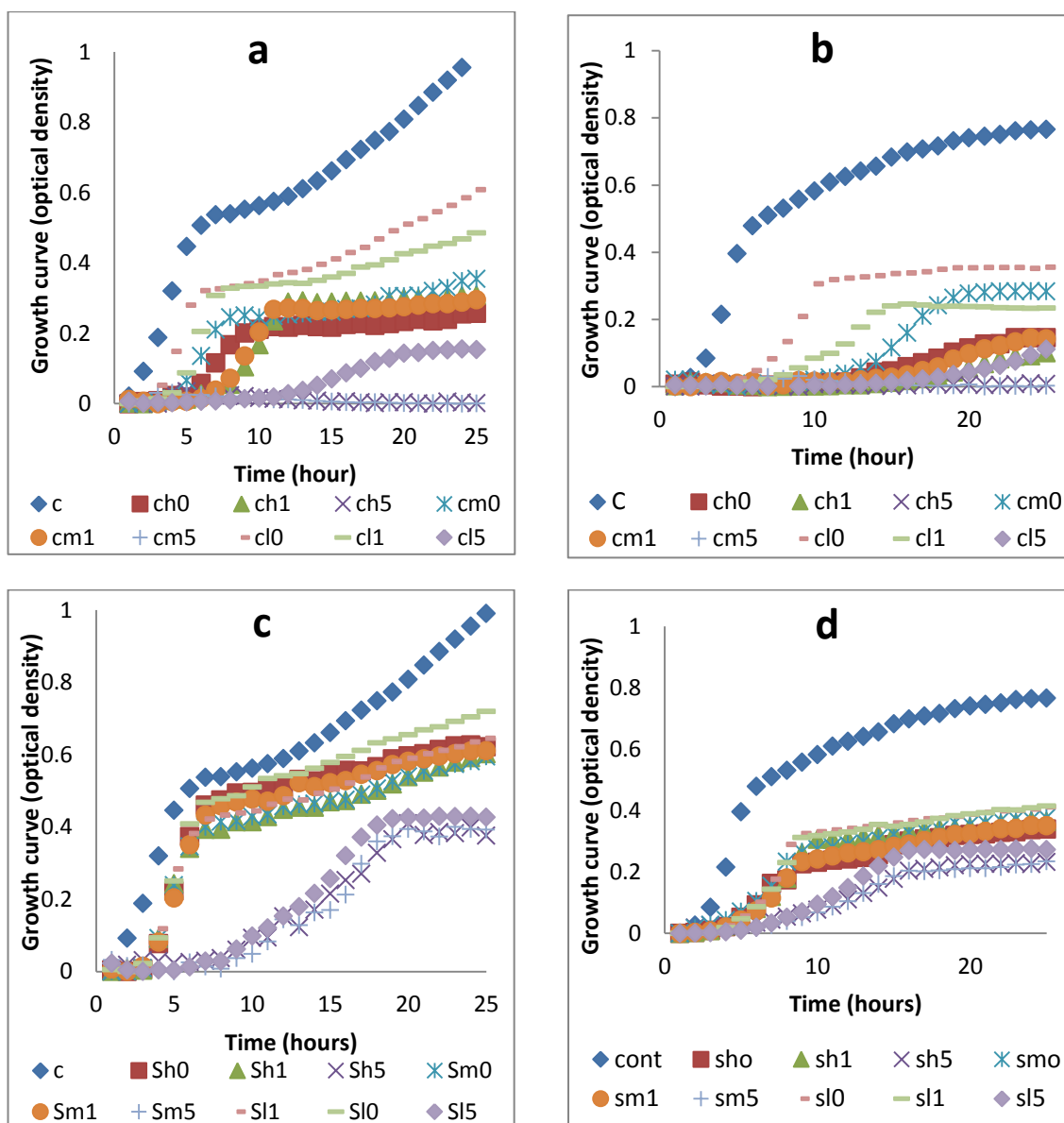


Colour change as result of spice extracts addition. C-: control negative, C+: control positive, Sh: sumac high (0.2), Sm: sumac medium (0.125), Sl: sumac low (0.05); Ch: cinnamon high (0.2), Cm: cinnamon medium (0.125), Cl: cinnamon low (0.05); Rh: rosemary high (0.2), Rm: rosemary medium (0.125), RL: rosemary low (0.05)

A.4 Data relevant for chapter (9)

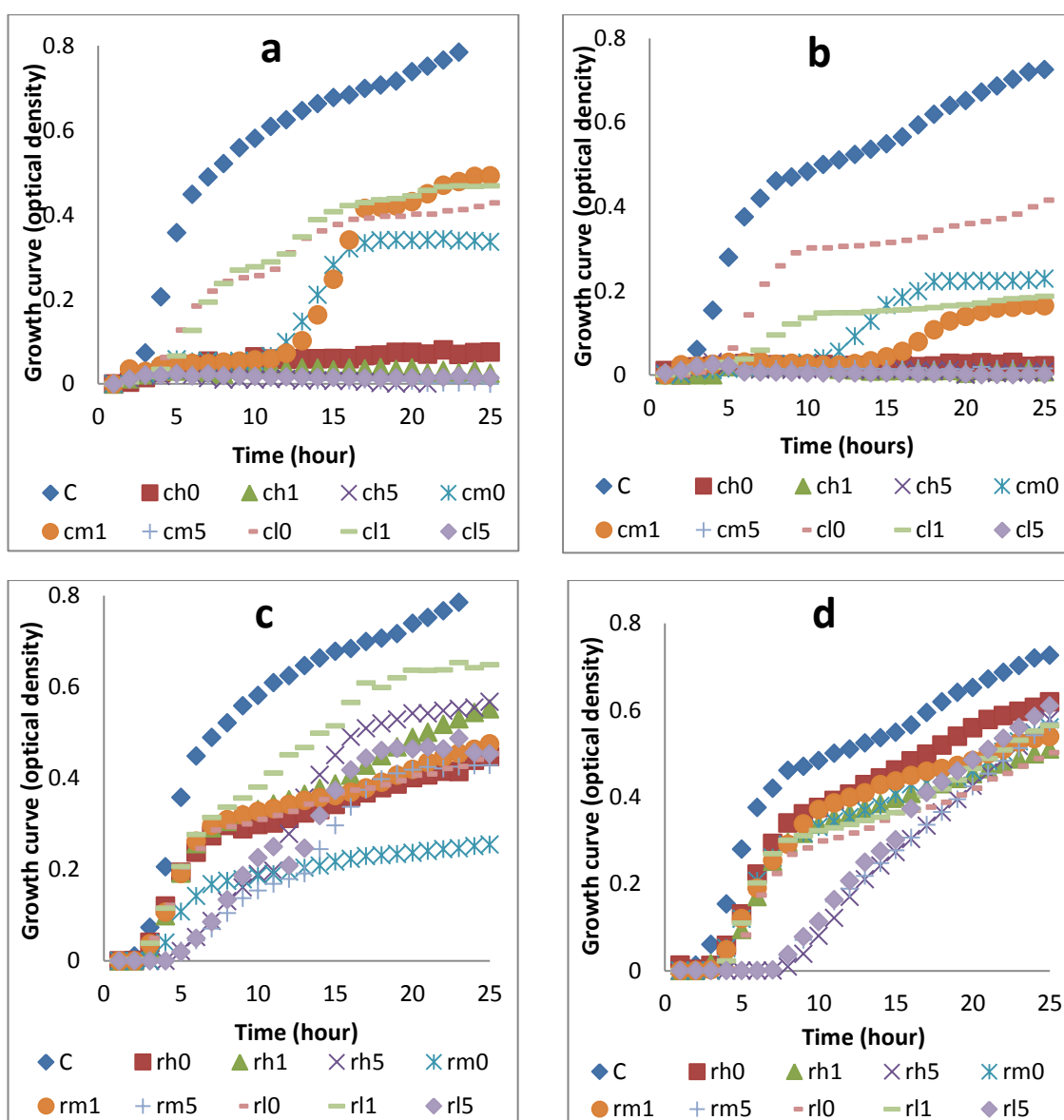


Growth curve of *Salmonella Typhimurium* DT104 (a, c) and *Listeria monocytogenes* (b, d) in broth culture (pH=7) at 30 and 15°C respectively using viable count measurements.



Growth curve of *Salmonella* Typhimurium DT104 (a, c) and *Listeria monocytogenes* (b, d) in broth culture (pH=7) at 30°C using plat reader.

C: control, **ch0:** cinnamon high concentration with 0% NaCl, **ch1:** cinnamon high concentration with 1% NaCl, **ch5:** cinnamon high concentration with 5% NaCl; **cm0:** cinnamon medium concentration with 0% NaCl, **cm1:** cinnamon medium concentration with 1% NaCl, **cm5:** cinnamon medium concentration with 5% NaCl; **cl0:** cinnamon low concentration with 0% NaCl, **cl1:** cinnamon low concentration with 1% NaCl, **cl5:** cinnamon low concentration with 5% NaCl, **sh0:** sumac high concentration with 0% NaCl, **sh1:** sumac high concentration with 1% NaCl, **sh5:** sumac high concentration with 5% NaCl; **sm0:** sumac medium concentration with 0% NaCl, **sm1:** sumac medium concentration with 1% NaCl, **sm5:** sumac medium concentration with 5% NaCl; **sl0:** sumac low concentration with 0% NaCl, **sl1:** sumac low concentration with 1% NaCl, **sl5:** sumac low concentration with 5% NaCl.



Growth curve of *Salmonella Typhimurium* DT104 (a, c) and *Listeria monocytogenes* (b, d) in broth culture (pH=6) at 30°C using plat reader.

C: control, **ch0:** cinnamon high concentration with 0% NaCl, **ch1:** cinnamon high concentration with 1% NaCl, **ch5:** cinnamon high concentration with 5% NaCl; **cm0:** cinnamon medium concentration with 0% NaCl, **cm1:** cinnamon medium concentration with 1% NaCl, **cm5:** cinnamon medium concentration with 5% NaCl; **cl0:** cinnamon low concentration with 0% NaCl, **cl1:** cinnamon low concentration with 1% NaCl, **cl5:** cinnamon low concentration with 5% NaCl, **rh0:** sumac high concentration with 0% NaCl, **rh1:** sumac high concentration with 1% NaCl, **rh5:** sumac high concentration with 5% NaCl; **rm0:** sumac medium concentration with 0% NaCl, **rm1:** sumac medium concentration with 1% NaCl, **rm5:** sumac medium concentration with 5% NaCl; **rl0:** sumac low concentration with 0% NaCl, **rl1:** sumac low concentration with 1% NaCl, **rl5:** sumac low concentration with 5% NaCl.

Appendix B

Sensory evaluation forms

B.1 Sensory Assessment of Doner Kebab

Date -----

Initials-----.

Please rate the sample of doner kebab of your acceptability on a scale from 1-7 where

1= Very Unacceptable

2= Moderately Unacceptable

3= Slightly Unacceptable

4= neither Acceptable nor Unacceptable

5= Slightly Acceptable

6= Moderately Acceptable

7= Very Acceptable

Please tick the corresponding box

| Character | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|--------------------|---|---|---|---|---|---|---|
| Aroma | | | | | | | |
| Texture | | | | | | | |
| Colour | | | | | | | |
| Overall appearance | | | | | | | |
| Overall acceptance | | | | | | | |

★ Please, warm the kebab sample for 15 second, in microwave (mild temperature) before judgment; thank you for your cooperation.

Please feel free to add any comment regarding your assessment of the sample here

B. 2 Sensory Evaluation Lamb Doner Kebab

Date -----

initials-----

Please rate the sample of doner kebab of your acceptability on a scale from 1-7 where:

- 1= Very Unacceptable
- 2= Moderately Unacceptable
- 3= Slightly Unacceptable
- 4= neither Acceptable nor Unacceptable
- 5= Slightly Acceptable
- 6= Moderately Acceptable
- 7= Very Acceptable

Please tick the corresponding box

| Attributes | Product code | | | | | | | |
|--------------------|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 765 | 059 | 087 | 233 | 294 | 944 | 526 | 098 |
| Colour | | | | | | | | |
| Texture | | | | | | | | |
| Overall appearance | | | | | | | | |
| Aroma | | | | | | | | |
| Overall acceptance | | | | | | | | |

Please warm up the sample before test using the microwave for 1 minute.

Please feel free to add any comment regarding your assessment of the sample here or overleaf.

Thank you.

B.3 Sensory Evaluation Lamb Doner Kebab (Ranking Test)

Panellist code:

Date: 11th/Dec/2009

Test: Doner kebab

Please evaluate each sample in the order that they are, starting from the left.

Please place all the samples in the order of ACCEPTABILITY for each one of the attributes below, one attribute at the time.

Please write the codes in increasing order of colour acceptability, overall appearance, etc. in the boxes below.

Once the first row is completed, proceed to the next one.

| Attribute | Least | Acceptability | | | | | | | | | | Most |
|--------------------|-------|---------------|--|--|--|--|--|--|--|--|--|------|
| Colour | | | | | | | | | | | | |
| Overall appearance | | | | | | | | | | | | |
| Aroma | | | | | | | | | | | | |
| Taste | | | | | | | | | | | | |
| Overall acceptance | | | | | | | | | | | | |

Please write the codes in increasing order of **spiciness** in the boxes below.

| Least | Spicy | | | | | | | | | | Most |
|-------|-------|--|--|--|--|--|--|--|--|--|------|
| | | | | | | | | | | | |

Please feel free to add any comment regarding your assessment of the sample here or overleaf
Thank you.

Appendix c

Poster presentations

Comparison of the Antibacterial Activity of Oil and Water Extracts of Culinary Spices

Sahar Al-Kutby, Jane Beal and Victor Kuri

Food, Nutrition & Health, School of Biological Sciences, University of Plymouth, United Kingdom



Background

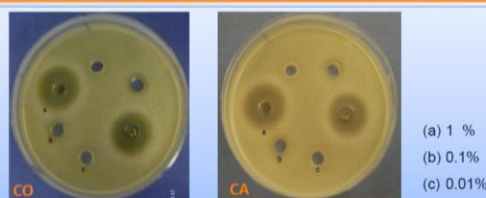
❖ Spices have been used as flavouring agents since ancient times to impart flavour, aroma or piquancy to foods in many areas of the world. Phytochemical compounds are present in spice extracts including: tannins, flavonoids, phenolic diterpenes, and phenolic acids. All of them are considered to be potential sources of natural antibacterial, antioxidant, anti-inflammatory and anticancer activity¹

❖ Food-borne diseases as well as food deterioration are considered to be a major problem. Following customer demands for wholesome foods, researchers and food processors are interested in controlling spoilage and preventing growth of pathogenic bacteria to obtain safe food of optimum quality, by the use of natural ingredients²

Aims

To screen two types of edible spice extracts (oleoresin & aquaresin) for antibacterial activity

To compare the efficacy of the extracts by determining the activity against a selection of pathogenic & spoilage bacterial strains



Materials and Methods

> Natural extracts

Rosemary, cinnamon, clove, black pepper and mint (R, C, CL, BP, M) were provided by (Kalsec, Mildenhall, UK) as aquaresin and oleoresin (A,O) which are water and oil dispersible respectively. Water and alcoholic extracts (A,O) were prepared from sumac (S) obtained from Green Cuisine Food products, Watton, UK.

> Bacterial strains

Ten food-borne pathogens and spoilage bacteria were used as target organisms: *Listeria monocytogenes* 5105, *Listeria innocua* 4202, *Lactobacillus sake* 550, *Staphylococcus aureus*, *Salmonella typhimurium* DT104, *Escherichia coli* K12, *Pseudomonas aeruginosa* B8626, *Bacillus cereus*, *Bacillus subtilis* and *Clostridium perfringens* NCTC8237. Strains were obtained from the School of Biological Sciences microbiological collection (University of Plymouth).

> Agar wells diffusion method³

Stock cultures of target strains were grown in nutrient broth for 18 h. Final cell concentrations were standardized to 10^7 – 10^8 CFU ml⁻¹ using the McFarland solutions standards (opacity tube⁴). 200 µl of these were added to each plate containing 20 ml brain heart infusion agar and left to set. Then wells (6 mm diameter) were formed in each plate using a sterile cork borer. Forty µl of each extract at different concentrations (1, 0.1, 0.01 w/v) were applied into each well and left to diffuse for 15–20 min at room temperature prior to incubation at the appropriate conditions.

> Determination of activity units (AU)

Sequential antimicrobial extracts two-fold dilutions with sterile saline were applied (40 µl aliquots) into the agar wells as above. The AU ml⁻¹ was the highest dilution with an inhibition diameter > 6 mm multiplied by a factor of 25. For the assay, inhibition was considered when no growth was detected after 24 h and the inhibition diameter was measured in 3 directions and the average was calculated. Three replications of this experiment were made⁵

Figure 1. Zone of inhibition for *Staphylococcus aureus* by cinnamon oleoresin (CO) and aquaresin (CA) at different concentrations

Results

Antibacterial activity of natural spice extracts were demonstrated to have different efficacy against pathogenic and spoilage bacteria depending on the type of the extracts and the concentration used. A 1% solution was significantly more effective ($p < 0.05$) followed by 0.1%, while 0.01% were found to be ineffective for all extracts (Table 1, Figure 1)

Table 1. Inhibitory properties^b of diluted spice extracts against pathogenic and spoilage bacteria^a

| Spice extracts | Bacterial strain | | | | | | | | | | |
|----------------|------------------|------|------|------|------|------|------|------|------|------|--|
| | L. m | L. i | S. a | S. t | E. c | B. c | B. s | P. a | L. s | C. p | |
| RO | ++ | ++ | ++ | 0 | 0 | ++ | ++ | 0 | 0 | ++ | |
| RA | ++ | ++ | ++ | 0 | + | ++ | ++ | 0 | 0 | ++ | |
| CO | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | 0 | ++ | |
| CA | + | ++ | + | + | + | + | + | ++ | 0 | ++ | |
| CLO | ++ | ++ | ++ | ++ | ++ | ++ | + | 0 | ++ | ++ | |
| CLA | + | + | + | + | + | + | + | 0 | + | ++ | |
| BPO | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | |
| BPA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | |
| MA | + | + | ++ | + | + | + | + | 0 | + | ++ | |
| SW | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | ++ | |
| SAC | ++ | ++ | ++ | ++ | ++ | ++ | 0 | ++ | ++ | ++ | |

^a Bacteria were tested at 10^7 CFU / ml for 24 h;

^b ++: Inhibition by 1/10 dilution of spice extracts;

+: Inhibition by 1/1 dilution of spice extracts;

0: No inhibition

References

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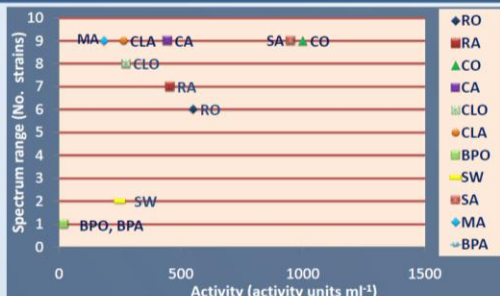


Figure 1. Spectrum range and activity (AU Means) of the spice extracts against selected bacteria

Mean values of activity units ml⁻¹ (AU) of spice extracts indicate that cinnamon oleoresin and sumac alcoholic extract were the most effective with AU about 1000 and 950 ml⁻¹ respectively. The moderate effect observed with rosemary, cinnamon aquaresin, clove, and sumac water extract was on the levels of 550, 455, 445, 275, 265, 250 and 185 ml⁻¹ respectively. Black pepper showed the lowest effect with 20 ml⁻¹ only and the smaller spectrum.

Conclusions

- Edible spice extracts have potential antimicrobial activity against pathogenic and spoilage bacteria
- Cinnamon, Sumac alcoholic extract have a strong efficacy against most bacterial strains tested with high level of activity
- Depending on the activity, Spice extracts could be useful for high fat content meat, and further experiments will include the application of the most effective extracts into ready-to-eat donner kebab

Acknowledgements:

SAK postgraduate scholarship by the Ministry of Science, Culture and Education of Iraq

To all the technicians at the food and nutrition & microbiological laboratories for support with the tests



Antimicrobial activity of spice extracts in ready-to-eat doner kebab on modified atmosphere package (MAP) storage at 4°C - challenge test against *Listeria monocytogenes*



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Background

- Listeria monocytogenes* (LM) is a gram-positive bacillus, facultative intracellular food-borne pathogen, ubiquitous in nature, readily contaminates meat products such as **DONER KEBAB**. It is very difficult to **INHIBIT** *Listeria monocytogenes* in Ready-To-Eat (RTE) products due to its ability to grow to significant numbers at refrigeration temperatures when given sufficient time. This limits the shelf-life of RTE foods.
- The vast majority of cases of listeriosis are associated with consumption of foods that do not meet current standards for *L. monocytogenes* in foods, whether that standard is zero tolerance or 100 CFU/g.
- Culinary spice extracts are well known for their antibacterial effects therefore researchers and food processors are focussing in controlling **LISTERIA** to obtain safe foods of optimum quality, by the use of natural ingredients.

AIM

To determine the effectiveness of antibacterial culinary spice extracts applied to modified atmosphere packed ready to eat doner kebab challenged with *L. monocytogenes* at 4°C storage

Materials and methods

Bacterial culture preparation

Two strains of *Listeria monocytogenes* NCTC5105 and NCTC7973, were prepared by incubation overnight in BHI broth at 37°C. The inoculum were prepared by mixing (1:1) the two strains culture previously adjusted to 10^7 CFU/ml

Natural spice extracts

Rosemary oleoresin (R), cinnamon oleoresin (C), and sumac alcoholic extract (S)

Sample preparation

Doner kebab was manufactured at the food and nutrition lab according to a conventional formula: 70% lamb meat, 28% lamb fat, Rusk 1%, salt 0.5%

Treatment 1 (Mix)

Spice extracts were added into the kebab before cooking, at high (H) and low (L) levels (0.025, 0.05ml/100g)



Treatment 2 (Dip)

After cooking, doner kebab slices were dipped into spice extracts at high and low levels (0.025, 0.05ml/100g)

Microbiological analysis

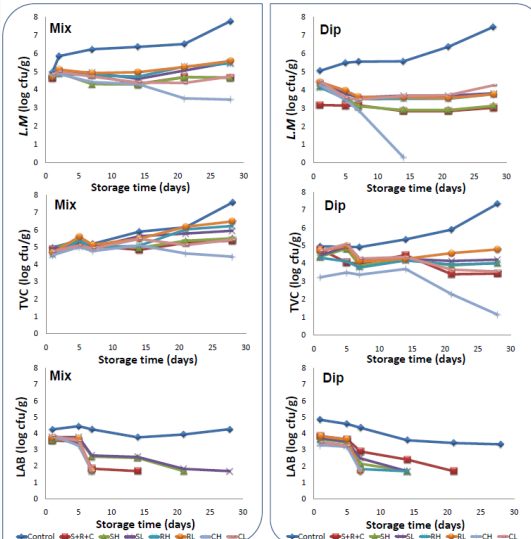
- L. monocytogenes* (LM) detection and enumeration followed health protection agency (2009) methods with Fraser, half Fraser broths and LSA agar
- Total viable counts (TVC) on plate count agar (PCA)
- Lactic acid bacteria (LAB) on MRS agar on CO₂

After cooking doner slices were inoculated with *Listeria monocytogenes* (10^5 CFU/g) and stored in MAP at 4°C

Results

Mix: spice added before cooking

Dip: spice added after cooking



Growth curves of *L. monocytogenes*, TVC and LAB in ready to eat doner kebab slices mixed or dipped with natural spice extracts

Conclusions

- A challenge test protocol against the growth of LM demonstrated that spice extracts could be useful to reduce the risk associated with RTE meat products for listeriosis
- The results confirmed that in the product, all spice extracts have bacteriostatic effects, while cinnamon oleoresin at high concentration have a potentially bactericidal effect against *L. monocytogenes*. Cross contamination results in low levels of bacterial population ($<10^4$), thus the use of spice extracts in meat products could maintain the bacterial population below the infectious dose, becoming an additional hurdle for risk reduction
- The type and the concentration level for spice extracts should be determined to achieve the desired Listeria control

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Thermal inactivation of spore forming bacteria demonstrated that sumac extract improve the safety of ready-to-eat doner kebab

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Background

- *Clostridium perfringens* and *Bacillus cereus*, are foodborne gram positive spore-forming rods which are ubiquitous in nature¹
- They are a concern to the foodservice industry² and *C. perfringens* has been the greatest food poisoning risk to public health associated with doner kebab³
- Food poisoning associated with these pathogens is due to their ability to form heat-resistant spores that survive commercial cooking processes
- Moreover, *C. perfringens* has the ability to grow rapidly at relatively high temperatures, adding concern when linked to RTE products
- Culinary spice extracts, such as Sumac (*Rhus coriaria*), have been found to have antibacterial properties⁴
- Doner kebab is commonly assembled in big pieces for cooking, where heating up and cooling down is relatively slow, and could allow spore-forming bacteria to grow

Aims

- To investigate the thermal inactivation of *B. cereus* & *C. perfringens* vegetative cells in RTE doner kebab
- To evaluate the antimicrobial effect of sumac extract

Materials and methods

Bacterial strains and enumeration media

B. cereus (NCIMB 11925): BCA and BCA over TCA
C. perfringens (NACTC 8239): OPSP and OPSP over TSA

Natural extracts

Hydro-alcoholic extracts were prepared from powder sumac spice, at 2% level

Inocula preparation

Strains inoculated in BHI broth, centrifuged (4000 g/10 min) to obtain cell pellets, washed and re-suspended in MRD, and standardised to 10^7 CFU ml⁻¹ for inoculation

Doner preparation

lamb meat 70%
rusk 1%
salt 0.5%

Sample preparation and inoculation

Sumac treated (and control) samples were cooked, cooled, sliced, and packed into polyethylene bags and vacuum sealed. Each 10 g sample bag was inoculated with 1ml 10^7 CFU ml⁻¹ of the *B. cereus* or *C. perfringens*. Triplicates samples were prepared

Thermal inactivation

Bags were fully submerged in water-baths at 50, 55 and 60 °C for *B. cereus* and at 55, 60 and 65 °C for *C. perfringens*, and bag samples were withdrawn at varying time intervals for bacterial enumeration

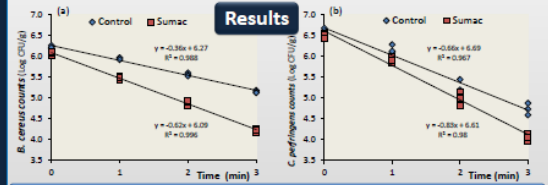
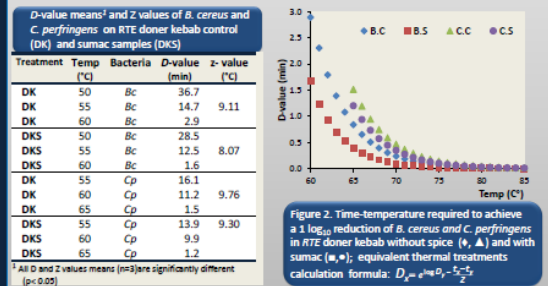


Figure 1. Survival curves for (a) *B. cereus* at 60°C and (b) *C. perfringens* at 65°C in RTE kebab



¹ All D and Z values means (ns) are significantly different ($p < 0.05$)

Conclusions

- Application of sumac could improve the safety of RTE doner kebab in regards to spore forming bacteria as the risk related to survival is reduced
- *B. cereus* and *C. perfringens* vegetative cell 6 log reductions could be achieved on control and sumac-treated RTE doner kebab, by heating at 70 °C for 1.4, 0.56 and 2.3, 1.8 min, respectively
- The cooking practices of RTE doner kebab could support the growth of spore forming bacteria
- Reformulating the product and verifying the effectiveness of suggested cooking protocols could be useful to assure the thermal inactivation of spore formers
- The data obtained from this study could be used in a future risk assessment studies

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Modelling antibacterial effect of spice extracts on growth of spoilage flora in VP and MAP cooked lamb product on chilled storage



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Background

- Predictive modelling is a promising field of food microbiology used to predict the likelihood and extent in the growth of spoilage and pathogenic bacteria under different physical or chemical conditions such as: temperature, pH, water activity, and preservatives¹
- Models could allow not only to predict the microbial safety or the shelf life of products, but also to determine the critical parts of the production and distribution process²
- Technologies such as vacuum packing (VP) and modified atmosphere packing (MAP) allow extended storage of ready to eat (RTE) meat products, but issues with chemical and microbial deterioration remain³
- Culinary spice extracts, such as Sumac (*Rhus coriaria*), have been found to have antibacterial properties⁴

Aims

To investigate the effect of natural spice extracts and packaging conditions on the growth kinetic parameter of spoilage indicators in ready to eat meat products during storage time at 4 °C

Materials and methods

Natural extracts

Rosemary oleoresin (R), cinnamon oleoresin (C) and sumac alcoholic extracts (S) at two levels (0.05% and 2%), and their combination (SRC) (1:1:1) (high concentration level) were used

Sample preparation

Samples were designed to resemble a RTE meat product with minced lamb and a cereal ingredient in a doner kebab formulation, prepared at a pilot plant. A base mixture was divided into 8 blocks of which 7 were treated with spice extracts as indicated above, while one control was left untreated. Each batches was homogenised separately in a bowl mixer with a spiral dough hook at 80 rpm for 5 minutes. After shaping, 10 x 5 cm slices were obtained with a 5 mm thickness

Cooking process

Kebab slices were cooked at 200 °C with a gas oven for 8 minutes to reach 72°C at the core



Storage conditions

Slices were VP and MAP (30% CO₂, 70% N₂) by using film made of polyamide and polyethylene sealing layer, the thickness was 70 µm

Microbiological analysis and modelling

TVC and LAB (log₁₀ cfu/g) were determined and data was fitted to the Baranyi and Roberts model with the DMfit v2 software

Conclusions

- Growth kinetic parameters from the Baranyi and Roberts model (DMfit) would enable technical practitioners to make rapid and realistic predictions for spoilage bacteria and shelf-life of convenience meats such as RTE doner kebab
- The use of cinnamon or rosemary significantly reduced the lag phase of TVC by about 2 weeks, and reduced the maximum final growth by 2 log cycles. All spices decreased LAB counts over time
- Application of spices in meat products offers viable alternatives for spoilage control and modelling aids the prediction of the shelf life and allows better targeting of formulation strategies

Acknowledgments

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Support by technicians at the food and nutrition & microbiological laboratories

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Results

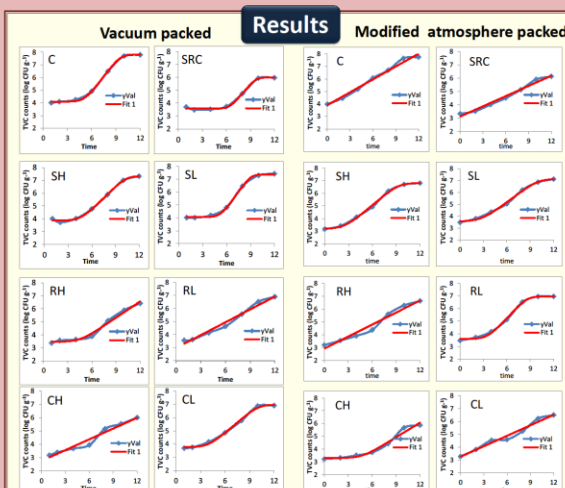


Figure 1. Total viable bacteria (TVC) counts (log CFU/g) in VP and MAP doner kebab slices with spice extracts, during storage time (weeks)

Key to Treatments: C:Control, SRC: Combination, SH: Sumac high, SL: Sumac low, RH: Rosemary high, RL: Rosemary low, CH: Cinnamon high, CL: Cinnamon low.

Table 1. Growth kinetics* for LAB in VP and MAP doner kebab with added spice extracts, during storage time obtained from Baranyi and Roberts model

| Packaging Type | Treatment | Modelling parameter | | | | | | | | | |
|----------------|-----------|---------------------|-------|------|------|------|------|---------|----------------|-----|--|
| | | mCurv | nCurv | rate | lag | y0 | yEnd | se(fit) | R ² | std | |
| VP | C | 10.00 | 1.00 | 0.83 | 4.0 | 4.10 | 7.80 | 0.08 | 0.990 | | |
| | SRC | 10.00 | 1.00 | 0.27 | 5.3 | 3.59 | 5.99 | 0.11 | 0.990 | | |
| | SH | 10.00 | 1.00 | 0.60 | 3.6 | 3.87 | 7.35 | 0.12 | 0.990 | | |
| | SL | 10.00 | 1.00 | 0.86 | 4.2 | 4.07 | 7.38 | 0.08 | 0.990 | | |
| | RH | 0.00 | 1.00 | 0.33 | 4.0 | 3.30 | 6.57 | 0.19 | 0.980 | | |
| | RL | 0.00 | 0.00 | 0.45 | 3.46 | 6.90 | 0.22 | 0.970 | | | |
| | CH | 0.00 | 0.00 | 0.26 | 3.13 | 6.08 | 0.25 | 0.940 | | | |
| | CL | 10.00 | 1.00 | 0.33 | 2.9 | 3.46 | 6.98 | 0.14 | 0.950 | | |
| MAP | C | 0.00 | 0.00 | 0.74 | 3.91 | 7.75 | 0.21 | 0.980 | | | |
| | SRC | 0.00 | 0.00 | 0.26 | 3.13 | 6.15 | 0.17 | 0.976 | | | |
| | SH | 10.00 | 1.00 | 0.53 | 2.0 | 3.20 | 6.81 | 0.11 | 0.990 | | |
| | SL | 10.00 | 1.00 | 0.59 | 2.0 | 3.59 | 7.14 | 0.13 | 0.992 | | |
| | RH | 0.00 | 0.00 | 0.31 | 2.93 | 6.63 | 0.29 | 0.956 | | | |
| | RL | 10.00 | 1.00 | 0.65 | 3.0 | 3.60 | 6.98 | 0.13 | 0.992 | | |
| | CH | 0.00 | 1.00 | 0.27 | 4.0 | 3.25 | 5.97 | 0.24 | 0.954 | | |
| | CL | 0.00 | 0.00 | 0.39 | 3.34 | 6.50 | 0.21 | 0.969 | | | |

* Primary parameters: rate: potential maximum growth rate, lag: lag time, y0: sigmoid curve start, yEnd: upper or lower asymptote of the sigmoid curve depending on growth trend; Curvature parameters: nCurv: at the beginning of the linear phase, mCurv: at the end of the linear phase, se(fit): Standard error of fitting, R²_stat: Adjusted R² of the fitting